Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 40, No. 6, June 1983

Volume 40, n° 6, juin 1983

Canada

Fisheries and Oceans
Pêches et Océans

Canadian
Journal
of Fisheries
and Aquatic
Sciences

Journal
canadien
des sciences
halieutiques
et aquatiques

Government of Canada
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Canadian
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Volume 40, No. 6, June 1983

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canadien
des sciences
halieutiques
et aquatiques

Volume 40, n° 6, juin 1983

Autumnal Habitat Shift of Juvenile Atlantic Salmon (*Salmo salar*) in a Small River

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RIMMER, D. M., U. PAIM, AND R. L. SAUNDERS. 1983. Autumnal habitat shift of juvenile Atlantic salmon (*Salmo salar*) in a small river. Can. J. Fish. Aquat. Sci. 40: 671–680.

Autumnal changes in behavior and distribution of three age-classes of juvenile Atlantic salmon (*Salmo salar*) were determined during 3 yr in the Little Sevogle River of northeastern New Brunswick. In summer, salmon were always observed above the streambed, each holding a station over a single, unshaded stone. About 84% of the entire population occurred in the run habitat-type, 12% in riffles and 5% in pools. Underwater visual censuses showed the salmon to be continuously numerous in summer, but, as soon as water temperature fell to or below 10°C in autumn, they disappeared from their stations and their visible population decreased by 92–98%. Thereafter, the salmon were found almost exclusively in sheltered substrate chambers beneath surface streambed stones. However, salmon distribution among runs, riffles, and pools (77, 18, and 5%, respectively) did not differ significantly from that in summer. Trapping, marking, and absolute population estimates indicated neither dwindling nor egress of the resident population. Thus, instead of suddenly leaving the river area or moving to another habitat-type at low temperatures, the salmon merely moved from their unsheltered summer stations to sheltered winter stations within the streambed. The results indicate the importance of winter habitat management in managing juvenile Atlantic salmon populations.

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Les changements, en automne, de comportement et de distribution de trois classes d'âge de saumons atlantiques immatures ont été déterminés sur une période de trois ans dans la

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Printed in Canada (J7091)
Imprimé au Canada (J7091)

Petite rivière Sevogle, dans le nord-est du Nouveau-Brunswick. En été, les saumons se trouvent continuellement au-dessus du lit du cours d'eau, chacun demeurant en place au-dessus d'une pierre non ombragée. Environ 84 % de toute la population se trouvent dans le type d'habitat de la remonte, 12 % dans les maigres et 5 % dans les fosses. Des recensements visuels sous l'eau démontrent que les saumons sont toujours nombreux en été, mais que, aussitôt que la température baisse à 10°C ou moins en automne, ils quittent leur poste et la population visible diminue de 92 à 98 %. Par la suite, les saumons se trouvent presque exclusivement dans des cavités abritées du substrat sous des pierres de la surface du lit. Cependant, la répartition des poissons parmi les remontes, les maigres et les fosses (77 %, 18 % et 5 % respectivement) ne diffère pas de façon significative de ce qu'elle était en été. Le piégeage, le marquage et les estimations de population absolue indiquent ni déclin ni sortie de la population résidante. C'est donc qu'au lieu de quitter soudainement le secteur de la rivière ou de changer de type d'habitat aux basses températures, les saumons ne font que se déplacer de leurs stations estivales non abritées à celles d'hiver, abritées, dans le lit du cours d'eau. Ces résultats font ressortir l'importance de la gestion des habitats d'hiver dans celle des saumons atlantiques immatures.

Received October 18, 1982
Accepted February 23, 1983

Reçu le 18 octobre 1982
Accepté le 23 février 1983

NUMEROUS studies have contributed knowledge of summer habitats of juvenile Atlantic salmon, *Salmo salar* (Elson 1967, 1975; Symons and Heland 1978), and other stream-dwelling salmonids (reviewed by White 1973 and Church et al. 1979). However, systematic field studies of winter behavior and ecology have involved only some species of trout and Pacific salmon (*inter alios*: Maciolek and Needham 1952; Needham and Jones 1959; Logan 1963; Chapman and Bjornn 1969; Bjornn 1971; Bustard and Narver 1975). Successful management programs require information from both seasons as there is no reason to assume that summer and winter behavior are the same in any species.

Various changes in the distribution and habits of juvenile Atlantic salmon have been seen in autumn and winter, but the observations have been few and inconclusive. Juveniles have been found to stay in their summer habitats in streams for the winter (Lindroth 1955; Smirnov et al. 1976; Karlstrom 1977; Riddell and Leggett 1981). More commonly, however, juveniles become scarce in shallows of small streams and difficult to net in autumn (Allen 1940; Saunders and Gee 1964; Gibson 1973), when they tend to move downstream (McCrimmon 1954; Stuart 1957; Saunders 1960; Meister 1962; Riddell and Leggett 1981), particularly as water temperature falls to 10−11°C (Stuart 1957) and lower (Riddell and Leggett 1981). The moving fish, including large parr (Riddell and Leggett 1981), presmolts (Stuart 1957; Meister 1962), precociously mature male parr (Saunders 1960; Riddell and Leggett 1981), and also age 0+ salmon (Saunders and Gee 1964), may either move from shallow to deeper and slower flowing parts of the streams (Allen 1940, 1941; Saunders and Gee 1964) or descend to larger streams (Riddell and Leggett 1981) and lakes (Stuart 1957).

Juvenile salmon appear to become quiescent below the critical temperature of 7°C (Allen 1940, 1941), but some activity may continue at even lower temperatures (Jones 1959; Riddell and Leggett 1981). That juveniles remaining in shallows for the winter may stay hidden among streambed materials is apparent from the increase in netting efficiency that follows disturbance of the streambed upstream of a net (Saunders and Gee 1964). Similar vanishing from view and occupancy of gravel interstices by juveniles has been observed in stream-tanks (Lindroth 1955; Gibson 1978) and is associated with falling water temperature in autumn; salmon become decreasingly evident at about 10°C with very few individuals remaining in view at 9°C (Gibson 1978).

Clearly, some translocation in streams and a change in activity or behavior of juvenile Atlantic salmon are likely at low temperature in either autumn or winter. Yet, it remains to be resolved definitively what proportion of a juvenile salmon population leaves the summer habitat, exactly where the salmon spend the winter, and whether the movements and changes in activity occur at a particular time and temperature. The objective of this investigation was to examine the amount, the time, and the temperature of autumnal movement and possible change in habitat requirements of juvenile Atlantic salmon. This was done by studying changes in their population size, behavior, and use of habitats in a riffle-pool environment of a small stream.

Materials and Methods

STUDY AREAS

Observations were made in a 5.6 km portion of the Little Sevogle River, a third-order tributary of the Northwest Miramichi River. The Little Sevogle River rises at an altitude of 800 m and flows for 39.5 km through mixed forest, entering the Northwest Miramichi River at sea-level.

Three study areas, sites A, B, and C, were selected which were very similar to one another and generally representative of the entire river. Table 1 shows the physical characteristics of these sites. Stream widths and depths were measured along transects across the river at 3-m intervals, depths being measured at 1-m intervals along each transect. To express substrate size, the longest exposed dimension was measured on 20 stones collected from the substrate surface along each of several transects crossing the river at 6-m intervals in sites A and B; substrate sizes were estimated by eye in site C.

In this study, riffles, runs, and pools were differentiated on the bases of water depth, water velocity, surface turbulence, and the amount of sediment on the surface of the substrate as follows:

TABLE 1. Physical characteristics of the Little Sevogle River at the study sites during summer and autumn, 1976–78.

Property		Study site	
	A	B	C
Distance to mouth (km)	9.3	6.9	7.1
Length (m)	135.0	129.0	150.0
Mean width (m)	8.6	11.6	8.5
Area (m^2)	1330.0[a]	1416.0[a]	1275[b]
Depth (cm) mean	37.0	32.0	39.0
maximum	99.0	114.0	110.0
Habitat-type (% area)			
riffle	26.0	24.0	25.0[c]
run	28.0	36.0	35.0[c]
pool	46.0	40.0	40.0[c]
Substrate material (%)			
bedrock	5.0	0.0	0.0[c]
boulder (>25.6 cm)	1.8	0.4	2.0[c]
cobble (6.4–25.6 cm)	58.8	61.3	55.0[c]
pebble (1.6–6.4 cm)	30.2	35.4	35.0[c]
gravel (0.2–1.6 cm)	4.2	2.9	8.0[c]

[a] Calculated by planimetry from constructed maps.
[b] Length × mean width.
[c] Estimated by eye.

	Riffles	Runs	Pools
Water depth (cm)	<25	25–45	>45
Surface water velocity ($cm \cdot s^{-1}$)	>40	20–40	<20
Surface turbulence	rough/broken	rippled	smooth
Substrate surface	no sand, silt, or detritus	no sand, silt, or detritus	much sand, silt, and detritus

Each study site had moderately dense streamside vegetation composed mainly of alder (*Alnus* sp.) and had regions of undercut banks. Rooted in-stream vegetation was rare in all study sites.

Fish species found in the study sites were: Atlantic salmon, American eel (*Anguilla rostrata*), blacknose dace (*Rhynichthys atratulus*), brook trout (*Salvelinus fontinalis*), common shiner (*Notropis cornutus*), lake chub (*Couesius plumbeus*), slimy sculpin (*Cottus cognatus*), threespine stickleback (*Gasterosteus aculeatus*), and the white sucker (*Catostomus commersoni*). Birds and mammals commonly observed in the study sites were the belted kingfisher (*Megaceryle alcyon*), common merganser (*Mergus merganser*), great blue heron (*Ardea herodias*) in summer, and mink (*Mustela vison*); the river otter (*Lutra canadensis*) was seen occasionally.

Atlantic salmon did not spawn in any of the study sites although extensive spawning areas existed directly upstream of all sites (50 m above site A, 325 m above site B, and 75 m above site C).

Ice began forming on the river during mid-November and a complete ice-cover existed from late November to mid-April. Frazil and anchor ice were never seen in the Little Sevogle River.

OPERATIONS

Fish were observed directly in all study sites during August and September 1976–77 and during August–November 1978 using a face mask, snorkel, and wet-suit. During the summer and early autumn, observation periods of 3-h duration were possible; but during the late autumn, observations were limited to 1–1.25 h by the low water temperature (0.1°C). Salmon did not respond visibly to the presence of a diver provided the diver moved slowly and carefully so as to create minimum disturbance.

Underwater visibility was about 8 m in bright sunlight whereas on cloudy days it was reduced to about 4 m. During spates, visibility was poor at less than 1 m and during these times underwater observations were not practicable. Observations in water depths greater than 15 cm were easily made and, because of the good visibility, all shallower areas could be seen easily from positions in deeper water.

A total of 120 h was spent in direct underwater observation of salmon. All notes were made immediately on an underwater writing board.

The relative abundance of salmon in all study sites was judged by taking visual censuses along transects. Under summer conditions, young Atlantic salmon are photopositive (Gibson 1965, 1966; Gibson and Keenleyside 1966) so a large proportion of a population can be expected to be readily visible to a diver. No attempt was made to scale the counts to represent absolute population sizes. In both site A and B, standard transects were established which zigzagged through the areas, taking care that all the various habitat-types in the sites were included on the transects. The transects were marked by permanent flags on the streambank and the same transects were used whenever a census was taken. The total transect lengths were 190 m in site A and 251 m in site B. A fish was counted if it lay within 1 m of either side of the transect. Thus, effective transect width was 2 m.

Censuses were taken only if the stream was well illuminated, the sky was at least 50% clear and stream flow permitted a 4-m visibility. The stream was entered downstream of the study site and the start of the first transect was approached. The transect was then carefully followed with frequent glances at the streamside flags. Salmon were counted and readily categorized into age-classes (0+, 1+, 2+) on the basis of size. As well, the general habitat-type, whether riffle, run or pool in which each fish resided was recorded. After the water temperature fell below 10°C, fish were difficult to find in the open stream and searches were then directed at crevices and spaces in the substrate, beneath undercut banks and in deadfalls. Each census required 45−50 min to complete. Sex and the presence of precocious males could not be determined visually so no separation on the basis of sex or maturity was made. The water temperature was recorded at the beginning and end of each census and the mean of the two temperatures was taken to be the temperature during the census. During the three field-seasons, 66 censuses were taken.

Estimates of the absolute abundance of salmon were obtained at sites B and C using the sequential removal method (Zippin 1956). Study sites were closed off at both ends by stop-nets and the fish between were removed during successive fishings using an electrofisher (200−600 V DC, 0.6−2.0 A). The site was fished from the lower to upper net over a period of 45−60 min. After a 30-min interval, the process was repeated. The procedure continued until the number of fish caught during successive fishings showed a distinct and large decline. Three to seven fishings were required for this to occur. The fish from each fishing were visually categorized into age-classes on the basis of size, counted, and placed into live-cars 25 m downstream from the lower net. Comparison of visual estimates of lengths and ages with measured lengths and scale read ages revealed a maximum error of 2% for visual estimation. After the last fishing, all fish were distributed between the stop-nets, duplicating as nearly as possible their previous distribution and after 1 h the nets were removed. The calculation of population sizes and errors was according to Zippin (1956).

To assess the amount of movement of salmon in the autumn, a trap spanning the entire river was placed at each end of study site B. Each trap was a double-ended 0.6-cm mesh fyke-type net which had a vertical partition so that it could catch fish from either end while maintaining the catches separately. Each end had a rubber extension on the bottom which was buried in the substrate. Wings, made from 0.6-cm mesh galvanized screening supported on iron pickets and with the lower edges buried in trenches dug in the streambed, reached from both ends of each trap to each shore. Thus, the stream was impassible to fish moving both up- and downstream.

The traps were inspected for fish at 08:30 and 19:30. All salmon leaving the study sites were anesthetized in a benzocaine solution (230 mg$\cdot$L^{-1}) and given a right pelvic fin clip. Salmon entering were anesthetized and distinctively marked subcutaneously with acrylic colors. After recovery, all fish were released in the direction they had been moving when caught. This provided a means of determining the degree of ingress and egress as well as the rate of passage through the study site.

An alternative approach was also taken to measure the degree of salmon movement from study site C. The total catch of the several electrofishings of any population estimate represented a large proportion of the total estimated population (80−90%). The total catch of one population estimate was anesthetized and batch-marked with a partial dorsal fin clip by trimming off the anterior point of the fin only enough to make it obvious on subsequent dates that the fish had been handled previously. After recovery, the fish were released between the stop-nets and the nets were removed 1 h later. The number of fish bearing a clipped fin was then recorded during subsequent population estimates.

SEQUENCE OF OPERATIONS

The individual operations outlined were carried out as follows. Censuses for relative abundance estimates were carried out on consecutive days in sites A and B on an approximately weekly schedule as weather and stream conditions permitted. In 1976, censuses were done during August 11−October 18. In 1977, they were done during August 16−September 26, and in 1978, during August 1−November 24. During September 1977, while water temperature was falling quickly, censuses were taken every 3 d in site B.

The traps were established at both ends of site B during the first week of September 1977, and fished continuously during September 6−28. During visual censuses in site B at this time, the traps were closed off for the 45−60 min required to do the census and opened half an hour later. On September 5, 1977, a population estimate was made in site B when water temperature was 16°C. At this time, the traps, which were closed off during electrofishing, served as stop-nets. Another population estimate was made on September 21 when water temperature was 8.5°C.

In 1978 a program of population estimates was used to measure movement in site C. Population estimates were made on August 19, September 12 and 16, October 16, November 3, 16, and 24. Corresponding visual censuses were made on August 18, September 11 and 20, October 16, November 2, 16, and 24. These operations indicated the relative and absolute numbers of salmon present in the site at these times. All fish captured during the September 12 population estimate were given dorsal fin clips.

STATISTICS

All comparisons of salmon distributions among riffles, runs, and pools were supported by tests for contingency log-likelihood (Zar 1974).

Results

BEHAVIOR IN SUMMER

A total of 3651 salmon was observed during the summers of 1976−78. The behavior of all age-classes was alike and not visibly affected by the habitat-types occupied. Usually, each salmon maintained a solitary apparently permanent, station on the riverbed at a depth of 6−66 cm. Mostly, the station

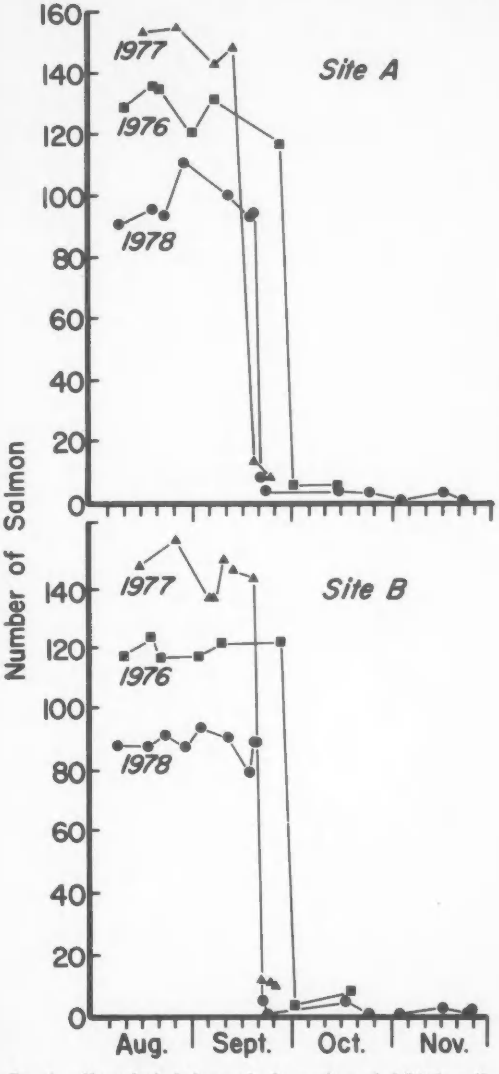

FIG. 1. Chronological changes in the numbers of visible juvenile salmon counted in sites A and B during 1976−78. Areas searched under transects during each census: A, 380 m²; B, 502 m².

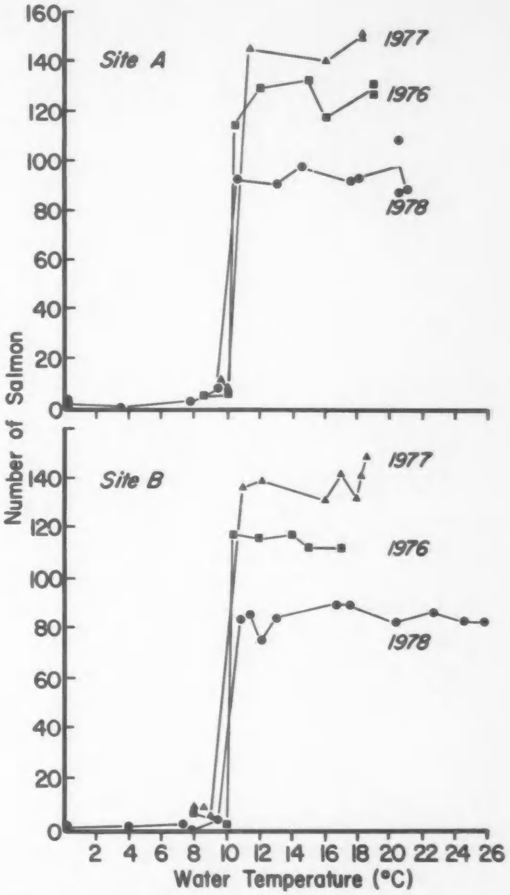

FIG. 2. Temperature related changes in numbers of visible juvenile salmon counted in sites A and B during 1976−78.

was over a single stone in unshaded sunlight. The salmon usually stayed quiescently in ventral contact with the stone while facing local water currents; however, some salmon alternated quiescence with brief periods of stationary swimming above their stones. The salmon made frequent, rapid dashes for current-borne prey; but they returned immediately afterward to their stations. Occasionally, a salmon would leave its station spontaneously and swim about for several minutes before returning. Few instances of agonistic behavior were noted. Streambed roughness probably isolated most of the largely sedentary salmon from their neighbors; nevertheless, up to five salmon were sometimes grouped in close association without evident aggressive displays. A salmon disturbed by the diver arched its back slightly, erected its dorsal fin and, frequently, turned to face the diver. A sudden movement of the diver at this time would cause the fish to move quickly upstream, curving away to one side and eventually returning downstream to a position beside its station. The station was occupied again after the diver withdrew. If a salmon was displaced gently by slow movements of the diver, it retreated for only a short distance and reoccupied its former position immediately after the diver had passed its station. It was notable that neither disturbed nor undisturbed salmon were ever seen to shelter among riverbed stones or any other shelter-type.

TABLE 2. Numbers of juvenile salmon counted visually along transects and estimated with the removal method in site B during September 1977.

Date	Temp. (°C)	Age (yr)	Number of salmon	
			Visual count	Removal method
Sept. 5	16.0	0+	111	178±33[a]
		1+	16	21±12
		2+	4	10±5
		All	131	203±23
Sept. 21	8.5	0+	7	155±42
		1+	2	20±3
		2+	0	10±7
		All	9	179±37

[a] ± 95% confidence interval.

SALMON DISAPPEARANCE

Salmon counted visually at sites A and B remained comparatively abundant until the end of each summer. Then, in late September or early October, their numbers always decreased greatly and with remarkable suddenness by about 92−98%, remaining consistently small for the duration of observations in the autumn. Figure 1 shows chronologically the changes in the visible populations of both sites. The abundance of the individual age-classes were similar.

Notably, the nearly complete annual disappearance of salmon always occurred during those periods of a few days when the mid-afternoon water temperature first fell to about 9−10°C. Thus, as is shown in Fig. 2, the salmon were still numerous at 10.5−11.2°C in site A and at 10.5−11.0°C in site B; but they were almost completely absent at 10.0°C and below. It became evident, therefore, that salmon population size was related to water temperature, with 10°C appearing critical to the sudden, great change in salmon numbers.

Although accurate censuses could not be taken in winter and spring because of ice, high runoff, and poor visibility, it appeared from general observations that salmon remained scarce throughout winter. In the spring of 1978, it was noted that no juvenile salmon were visible at either site before mid-May at which time the 1+ and 2+ age-classes appeared abruptly when mid-afternoon water temperature rose above 10°C. After emergence of the 0+ age-class from incubation gravels in mid-June, the newly established population did not markedly change size again before September.

Temperature change in autumn had no apparent effect on the absolute number of salmon in site B during September 1977 as the estimated population size, consisting of 203 fish at 16.0°C on September 5 and of 179 fish at 8.5°C on September 21, did not change significantly (Table 2). In contrast, the number of salmon counted visually, equalling 131 fish on September 5 (and remaining undiminished until September 19 at 11.0°C), diminished to 9 fish by September 21 (cf. Fig. 1). Since the absolute number of resident salmon remained essentially unchanged, even after the number of visually counted salmon decreased greatly, the disappearance of salmon was clearly not caused by mortality or by large egress of salmon from the sites.

The results of the trapping experiment at site B were in agreement with the overall stability of the absolute population size at the site. Salmon were caught discontinuously on only 14 of the 22 d when the traps were in operation. Of the 24 salmon caught leaving the site downstream, 11 left during September 6−14, well before the disappearance of the visible salmon and while the temperature was at least 12.2°C, 3 left when temperature was above 10°C on September 20, and the final 10 left when temperatures were below 10°C during September 23−27. Six salmon entered the area from downstream, three entering during September 7−20 and three during September 23−25. One salmon entered from upstream on September 27. The moving salmon appeared not to reverse direction or continue through the site as none of the trapped, marked individuals was recaptured at either end of the site. Thus, a total of 25 salmon moved downstream and 6 moved upstream within a 129-m length of the river; net egress amounted to only 17 salmon in 22 d, the population remaining largely static. The slight amount of downstream movement was clearly insufficient to account for the disappearance of visible salmon from the site and apparently unrelated to either the time or the temperature critical for disappearance (Fig. 3). The movements could, however, serve as an indication of possible autumnal translocation of some resident salmon within and near the site.

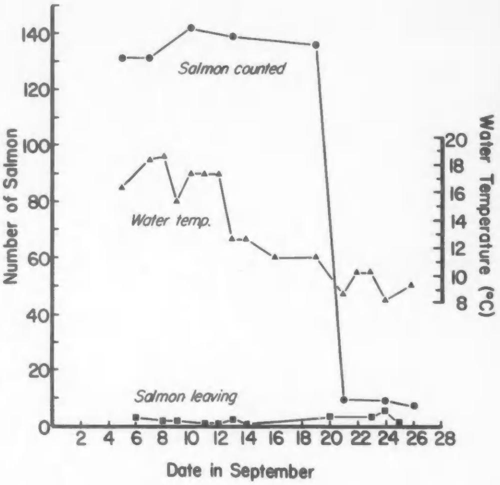

FIG. 3. Water temperature, visible numbers of juvenile salmon counted on transects in site B, and numbers of salmon leaving site B caught in traps during September 1977.

BEHAVIOR IN AUTUMN

During thorough underwater searches of all possible hiding places along transects in sites A and B on scheduled census days during September 20−26, 1977, and again during September 21−November 25, 1978, 168 juvenile salmon of all age-classes were found in all streambed areas where they had been common in summer. They were still most numerous in the runs which were occupied by 76.8% of the population found on transects. Riffles and pools were occupied by only

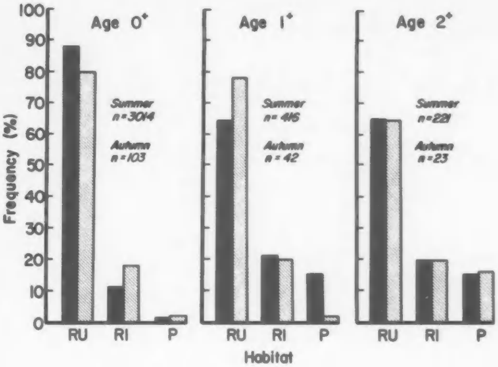

FIG. 4. Summer (black) and autumn (hatched) occupancy by three age-classes of juvenile salmon seen in runs (Ru), riffles (Ri), and pools (P) in sites A and B during 1977–78.

19.1 and 4.2% of the population, respectively. Now, salmon always resided solitarily in natural chambers beneath some of the cobble and boulders lying on the streambed surface. The chambers were closed on all sides, except for at least one distinct opening large enough for a salmon to pass through. There was no apparent relationship between the position of the openings and the direction of stream flow. Chamber size was just sufficient to hold one salmon. Some loose detritus and sediment had accumulated in the chambers, but salmon were not buried or wedged in the space. After the top stone was lifted from a chamber, the exposed resident salmon remained motionless for several seconds before swimming quickly upstream, very close to the streambed. The movement, consisting of a series of rapid darts and probing motions directed at the streambed, ended within 1–2 m as the salmon again disappeared into the streambed. It is possible that a substantial number of the small salmon in the sites, especially those of age 0+, escaped being counted because they could evade the diver by moving away unseen through very small spaces within the substrate. Nonetheless, in contrast to their behavior during summer at temperatures above 10°C, the salmon now showed a strong tendency to stay in shelter.

HABITAT OCCUPANCY

The relative frequency distribution of juvenile salmon, inclusive of all ages, among riffles, runs, and pools at and below 10°C during autumn did not differ significantly ($P > 0.05$) from their distribution at higher temperatures during summer. In both summer and autumn, all age-classes were markedly more frequent in runs than in either riffles or pools (Fig. 4). This order of habitat occupancy was remarkably persistent. The distribution of any age-class did not differ significantly ($P > 0.07$) from year to year and site to site and significant differences did not arise between the summer and autumn distributions of either age 0+ ($P > 0.06$) or 2+ ($P = 0.95$) salmon. However, a significant difference ($P < 0.025$) did exist between the summer and the autumn distribution of 1+ salmon. The difference was attributable to significantly decreased occupancy of pools in the autumn (subdivided contingency table — runs and riffles without pools: $P > 0.50$; pools versus runs and riffles combined: $P < 0.025$) and, since the occupancy of runs increased at the same time, this suggests an autumnal translocation of some age 1+ salmon from pools to runs.

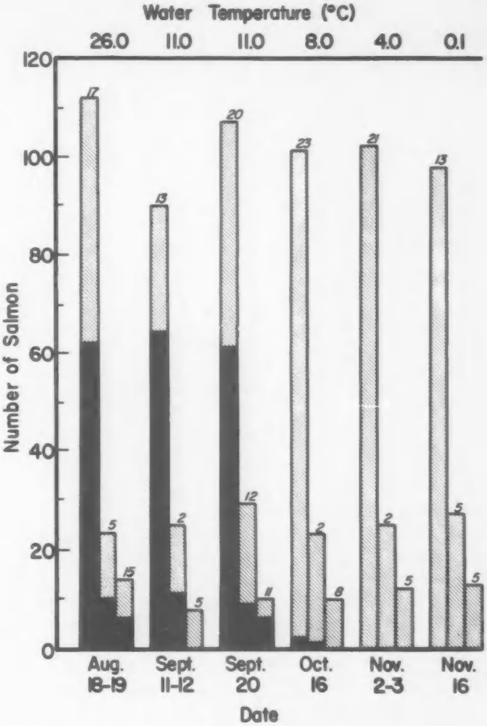

FIG. 5. Numbers of juvenile salmon in site C during August–November 1978. Hatched columns are estimated absolute population sizes (± 95% confidence interval in numerals). Black columns are counted visible population sizes. For each date, the left, middle, and right columns show ages 0+, 1+, and 2+, respectively.

The occurrence of juvenile salmon in pools was apparently age-dependent. Thus, age 0+ salmon were almost entirely absent from pools during both summer and autumn whereas some 15% of age 1+ salmon occupied the pool environment as summer residents. More permanent all-year residence of pools by a substantial part of the population became manifest only at age 2+.

Because salmon were found persistently in the same general habitats in both summer and autumn, it was clear that the advent of low autumn and winter temperatures had not caused large numbers of the population to abandon their usual areas of summer residence. Instead, many salmon probably had only removed themselves from the exposed macroenvironment in autumn and were exploiting the sheltered microenvironment available locally within the streambed.

TABLE 3. Proportion of juvenile salmon population, marked and released at 11.0°C on September 12, 1978 in site C, remaining on subsequent dates. Numbers marked: 80 age 0+, 24 age 1+, 5 age 2+.

			Fish captured			
				Marked fish recaptured		
Date	Temp. (°C)	Age (yr)	Total number	Number	% of total number captured	% of number marked
Sept. 20	11.0	0+	90	70	77.8	87.5
		1+	24	21	87.5	87.5
		2+	7	5	71.4	100.0
Oct. 16	8.0	0+	85	71	83.5	88.7
		1+	22	19	86.4	79.1
		2+	8	5	62.5	100.0
Nov. 3	4.0	0+	84	67	79.8	83.7
		1+	23	21	91.3	85.8
		2+	9	5	55.5	100.0
Nov. 16	0.1	0+	82	68	82.9	85.0
		1+	25	22	88.0	91.7
		2+	10	5	50.0	100.0

LACK OF EMIGRATION

Although sheltering in the substrate was indicated as the primary cause of the autumnal disappearance of visible salmon, egress of at least some salmon from the sites could not be ruled out entirely. Therefore, the potential importance of egress in salmon disappearance was investigated more conclusively during 1978 in site C. Visual censuses were taken and absolute populations in the site were estimated from mid-August until ice formation in late November. Salmon movement during mid-September to November was examined with the mark−recapture method, instead of trapping.

Until September 20, with water temperature at and above 11°C, visual counts remained almost constant at 76−83 salmon per census. After that date, in water of 0.1−8.0°C, the visual counts decreased markedly and remained consistently low at 0−3 salmon per census. However, the absolute number of salmon present in the site ranged between 120 and 147 without a marked decrease after September 20. Figure 5 shows also that the three age-classes of salmon were essentially alike in the decrease of their visible numbers as well as in the stability of their absolute numbers. An additional census was taken on November 24 when at 0.1°C and under ice that covered more than half the river surface, one age 0+ salmon was recorded on the transects. Use of the removal method was unsuccessful because ice restricted proper handling of both the electrofisher and the stop-nets.

The mark−recapture experiment, started on September 12 while water temperature was 11.0°C, revealed that 79.1−100% of the marked salmon stayed in the site until the end of the experiment on November 16 (see "% of number marked" in Table 3), when temperature had fallen to 0.1°C. That 0+ and 1+ salmon in and near the study site did not move, but remained essentially static throughout the period, is deducible from the persistently high percentages (77.8−91.3) of marked individuals recaptured ("% of total number") among these age-classes caught on each date. Although the five initially marked 2+ individuals were always recaptured, the percentage of marked 2+ salmon in the population decreased progressively while their total number captured increased to 10 by November 16. Thus, some unmarked age 2+ salmon appeared to enter the site from elsewhere, but not in significant numbers (cf. 8 ± 5 age 2+ salmon on September 12 and 13 ± 5 on November 16).

Discussion

Apparent decrease of juvenile Atlantic salmon populations in autumn have been noticed during studies of other phenomena. Allen (1940) reported the disappearance of salmon from riffle areas and a general decrease in netting efficiency at a water temperature of 7°C. Saunders and Gee (1964) found that stream areas which had produced good summer seine catches of young salmon yielded either few or no fish during repeated seining attempts after late September. Gibson (1973), by counting fish underwater in a large river, found that salmon numbers fell sharply in late September at a water temperature of roughly 12°C. Subsequently, Gibson (1978) found that, in a stream tank, young salmon were less evident when water temperature fell to about 10°C, and at 9°C very few fish could be seen. Although these studies demonstrated that large changes could occur in apparent population sizes or distributions of young salmon with the coming of winter, the suddenness with which these changes occur and the high temperature at which they occur have not been fully appreciated.

The stability of population numbers before and after the apparent population decline and the rapidity of the decline suggests that some threshold in the response to the environment had been passed. The consistency with which the behavior and the habitat occupied by the fish changes at 10°C suggests that the threshold is temperature-related, although the very close relationship between declining water temperature and photoperiod at this time of year makes it difficult to separate the effects of the two regimes. Chapman and Bjornn (1969) and Bjornn (1971) showed that under constant photoperiod, the number of young steelhead trout (*Salmo gairdneri*) hiding was negatively correlated with water temperature. In the present study, the fish observed during 1976 vanished from sight approximately 10 d later than those observed during 1977−78 when the water temperatures were slightly cooler during late September than in 1976 (cf. Fig. 1 and 2). Since the water temperature at which the fish disappeared was always the same while the time was not, water temperature may play a greater role than photoperiod in initiating the sudden change in behavior and habitat choice. This is not to say that photoperiod plays no role; the synchrony of photoperiod and temperature cycles would serve as a mechanism ensuring that a decline in temperature was not sensed as a chance occurrence, but as a part of general autumnal cooling.

The population declines associated with water temperature falling below 10°C in the Little Sevogle River have been shown to be only apparent. The total number of salmon leaving site B and caught in the traps during September 1977, (27) is far too small to explain the 94−98% declines in population size that appear in the visual census results. Also, the trapping results and population estimates in site B, supported by the population estimates and marking experiment in site C, showed not only that the actual population size remained stable while the visible population declined, but also that a large proportion of the salmon population stayed in the area after the apparent decline occurred.

This view is supported by several other studies. Saunders and Gee (1964) reported that while juvenile salmon were more difficult to find in autumn than in summer, capture success increased after stirring up the stream bottom upstream of the seine net. In stream tank studies, Lindroth (1955) noted that young salmon sought cover in the bottom gravel during winter and Gibson (1978) reported that this occurred when water temperature fell below 10°C.

Despite reports of sedentary populations in autumn, some have shown sizeable up- and downstream movements in the autumn. However, these movements can be associated with unusual situations ranging from opportune juxtaposition of streams and reservoirs (Stuart 1957), precocious sexual development (Jones and King 1949; Saunders 1960; Riddell and Leggett 1981), and imminent smoltification (Meister 1962; Riddell and Leggett 1981).

Chapman and Bjornn (1969) and Bustard and Narver (1975) found both movement from and hiding in an area to occur together in various species of Pacific salmon and trout. It is probable that this happens with Atlantic salmon as well. Although 79.1−100% of the juvenile salmon in the Little Sevogle River marked within site C stayed there during the apparent population decline, the approximate 50% decline in the proportion of marked 2-yr-olds between September 12 and November 16 indicates an influx of 2-yr-olds from elsewhere. Because this study has shown that all age-classes seek shelter in substrate chambers, the additional fish may have been those unable to find suitable areas in the substrate in areas of their summer habitat and that had moved or were moving to suitable areas. Chapman (1966) suggested that the primary population density regulator may change from being space and food-oriented in the summer to solely space-oriented in the winter. Probably, the larger fish (i.e. the age 2+ fish) would be those to leave an area possessing a limited size-range of interstices in the substrate to be used as winter chambers.

The persistently conspicuous disappearance of salmon, always clearly associated with a critical decrease in water temperature to below 10°C, but not discernably with salmon movements, resulted from sheltering alone. Sheltering in chambers must, therefore, be seen as a distinct and mandatory response to autumn, evokable independently of salmon movements and emigration.

During both summer and autumn, each age-class was found mainly in the runs, secondarily in the riffles and least in the pools. This order of occupancy indicates that habitats were selected and does not simply reflect habitat-type availability (cf. Table 1, Fig. 4). This agrees generally with past studies insofar as, in summer, juvenile salmon inhabit the comparatively shallow, fast-flowing, and unsilted areas of streams. Comparative information on salmon habitat occupancy during cold weather is scarce and inconclusive. Whereas Allen (1940, 1941), Saunders and Gee (1964), and Riddell (1979) suggest that salmon occupy pools during the winter, Smirnov et al. (1976) and Karlstrom (1977) suggest that salmon occupy the same habitat in both summer and winter. Past discrepancies may be attributable to indirect and disruptive sampling methods (i.e. seines, electrofishers). Direct underwater observation as performed in the Little Sevogle River has provided unbiased evidence that autumnal movement to pools did not occur and is, thus, not mandatory for juvenile Atlantic salmon. It would, in fact, be disadvantageous to do so because the silted substrate in pools would restrict the availability of substrate chambers which this study has shown juvenile salmon to occupy exclusive of all other shelter-types. This suggests that the habitat occupied in cold seasons may be related to the shelter-type required. Bustard and Narver (1975) found significant negative correlations between water temperature and water depth occupied by juvenile coho salmon (*Oncorhynchus kisutch*) and yearling steelhead trout which sheltered among upturned roots, sunken logs, and debris, all of which are associated with deep pools. However, underyearling steelhead trout, like juvenile Atlantic salmon, sheltered beneath the substrate material and no temperature−depth correlation existed for this age-class. Therefore, it appears that species, or age-classes, which shelter beneath substrate material do not move into pools as do those which use other shelter-types.

The magnitude of the sheltering response and the consistency with which it occurred demands that serious consideration be given to the management of the winter habitat as a means of managing juvenile Atlantic salmon populations. It is particularly germane in this regard that Gilbert (1978) found increases in the population densities of all age-classes of juvenile salmon following addition of large rocks (40−80 cm) to the substrate of the Big Tracadie River, N.B. This is probably the correct means of winter habitat management for this species. Because this species uses substrate chambers exclusively, using traditional habitat development and protection methods such as adding deadfalls, streamside cover, digger logs, deflectors, etc. to provide winter shelter would probably not make a great contribution to the suitability of a stream as winter habitat. Nonetheless, the disastrous consequences of not considering winter habitat management is illustrated by Mason (1976) who found that large increases in coho salmon biomass and potential smolt yield, obtained by supplemental summer feeding (summer habitat management), were nullified by the limited natural winter carrying capacity of the stream. Glova and Mason (1977) showed that winter carrying capacity was restricted by lack of shelter.

Acknowledgments

We gratefully acknowledge the financial support provided by the National Research Council of Canada (now NSERC), the University of New Brunswick School of Graduate Studies and Research, the O'Brien Foundation, the World Wildlife Foundation of Canada, the Miramichi Salmon Association and the University of New Brunswick President's Salmon Fund. Valuable advice was offered by P. F. Elson. The Zoology Department, University of Otago, Dunedin, New Zealand provided support and advice during manuscript preparation.

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An Energetics Model for Lake Trout, *Salvelinus namaycush*: Application to the Lake Michigan Population[1]

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An energetics model is implemented for lake trout, *Salvelinus namaycush*, and applied to the Lake Michigan population. It includes an egestion function allowing any proportional mix of fish and invertebrates in the diet, a growth model accounting for both ontogenetic and seasonal changes in energy-density of predator and prey, a model for typical in situ swimming speed, and reproductive energy losses due to gametes shed. Gross conversion efficiency of energy by lake trout over their life (21.8%) is about twice the efficiency of converting biomass to growth because they store large amounts of high-energy fats. Highest conversion efficiencies are obtained by relatively fast-growing individuals, and over half the annual energy assimilated by older age-classes may be shed as gametes. Sensitivity analysis indicates a general robustness of the model, especially for estimating consumption by fitting a known growth curve. Largest sensitivities were for the intercept and weight dependence coefficients of metabolism. Population biomass and associated predatory impact of a given cohort increase steadily for about 3.5 yr then decline steadily after fishing mortality becomes important in the fourth year in the lake. This slow response time precludes manipulation of lake trout stocking densities as a means to control short-term prey fluctuations. Predation by lake trout on alewife, *Alosa pseudoharengus*, has been increasing steadily since 1965 to about 8 400 $t \cdot yr^{-1}$, and is projected to rise to almost 12 000 $t \cdot yr^{-1}$ by 1990.

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On a élaboré un modèle de l'énergétique à l'intention du touladi, *Salvelinus namaycush*, que l'on a appliqué à la population du lac Michigan. Le modèle inclut une fonction d'égestion permettant d'introduire dans le régime alimentaire un mélange proportionnel de poissons et d'invertébrés, un modèle de croissance qui tient compte des changements à la fois ontogéniques et saisonniers de l'énergie et de la densité du prédateur et de la proie, un modèle de vitesse de nage in situ typique et, enfin, la perte d'énergie reproductrice par suite de la mise en liberté de gamètes. Comme le touladi emmagasine de grandes quantités de graisse de haute énergie, le rendement nutritif brut durant toute la vie du touladi (21,8 %) est environ le double de celui de conversion de la biomasse en croissance. On obtient des rendements maxima avec des individus à croissance relativement rapide, et plus de la moitié de l'énergie accumulée par les poissons plus âgés peut être libérée sous forme de gamètes. D'après une analyse de sensibilité, le modèle est généralement solide, surtout lorsqu'il s'agit d'estimer la consommation en adaptant une courbe de croissance connue. Les sensibilités les plus importantes touchent l'ordonnée à l'origine et les coefficients de métabolisme dépendant du poids. La biomasse de la population et l'impact de prédation qui lui est associée d'une cohorte

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Printed in Canada (J5995)
Imprimé au Canada (J5995)

donnée augmente régulièrement pendant environ 3,5 ans, pour ensuite diminuer de même une fois que la mortalité par pêche est devenue importante dans le lac pendant la quatrième année. À cause de cette lente réponse, on ne peut manipuler la densité des peuplements de touladis d'un lac comme moyen de contrôle à court terme des fluctuations des proies. La prédation du touladi sur le gaspareau, *Alosa pseudoharengus*, a augmenté régulièrement depuis 1965 pour atteindre environ 8 400 tm·an^{-1} et on prévoit qu'en 1990, elle aura atteint presque 12 000 tm·an^{-1}.

Received March 5, 1980
Accepted February 23, 1983

Reçu le 5 mars 1980
Accepté le 23 février 1983

THE lake trout (or charr), *Salvelinus namaycush*, has historically been a dominant top predator as well as an important component of the commercial catch in Lake Michigan. Extinction of wild populations in the mid-1950s and reestablishment of a hatchery-dependent (nonreproducing) lake trout population by the early 1970s is well-known (Smith 1968; Wells and McLain 1973; Rybicki and Keller 1978). The existing population supports an important sport fishery. Managers are being pressed by public enthusiasm to increase salmonid stocking densities, but increases cannot continue indefinitely because salmonid production in Lake Michigan must necessarily be limited by available production of forage organisms.

This study uses bioenergetics modeling to address a fundamental management question: "What are the forage requirements of existing and projected future lake trout populations in Lake Michigan?" It brings together subsets of data from studies conducted at the Great Lakes Fishery Laboratory (GLFL, USFWS) (D. Rottiers and associates, unpublished data summarized in Research Completion Reports; G. Eck, personal communication, GLFL, Ann Arbor, MI), available literature, and newly developed energetics theory. The approach begins with a mass-balance equation (Winberg 1956; Warren and Davis 1967; Kitchell et al. 1977; Webb 1978), and is the latest in a series (e.g. Kitchell et al. 1974; Kitchell et al. 1977; Weininger 1978; Breck and Kitchell 1979; Kitchell and Breck 1980). New functions are developed for egestion, swimming speed and growth with ontogenetic and seasonal changes in energy density of predator and prey.

Our objective is to reconstruct food consumption from observed growth rather than to predict growth. Observed growth in Lake Michigan results from consumption integrated over time. The summation of daily growth, metabolism, and waste losses provides an estimate of daily consumption for the average individual lake trout. Given the existing data base on the energetics of lake trout, we believe the energetics approach may be the most cost-effective and perhaps the most accurate of available methods (Mann 1978) for estimating annual forage needs of the trout. Forage requirements of the average individual are extended to the entire population using a population model with mortality rate estimates from Rybicki and Keller (1978). Absence of natural reproduction in the Lake Michigan population simplifies population biomass estimates, since known recruitments come from hatcheries.

Components of the Energetics Model

ENERGETICS BALANCE AND MAXIMUM CONSUMPTION

Our energetics model is based on the assumption that the sole energy source for an animal is its food (C, consumption), and that all energy taken in must be used (R, metabolism; SDA, specific dynamic action), lost (F, egestion; E, excretion), or accumulated (ΔB, growth). These components form the energetics balance:

$$C = R + SDA + F + E + \Delta B$$

All components can be converted between mass and energy units. In the remainder of this section the relationship of these components to their controlling variables are developed.

Growth must necessarily be less than or equal to that attainable at maximum consumption. In modeling simulations, laboratory estimates of maximum consumption are adjusted downward until the model fits observed growth (see below). Ad libitum weight-specific consumption $C_{\max}$ (g·g^{-1}·d^{-1}), increases exponentially with increasing temperature at low temperatures, reaches a maximum level at some optimum temperature, and decreases at temperatures greater than optimum (Brett et al. 1969; Edsall et al. 1974; Elliott 1975a, b). For low temperatures, $C_{\max}$ can be represented as a negative power function of weight (Brett 1971a; Kerr 1971; Elliott 1976b), and an exponential function of temperature (Elliott 1976b):

$$(1) \quad C_{\max} = a\, W^{b} \cdot e^{qT}$$

where, W = fish weight, g
T = temperature, °C
and a, b, q = empirical constants.

This simple model differs from our previous work (Kitchell et al. 1977) but may be appropriate for the temperature range which a lake trout typically occupies of its own volition.

The parameters of eq. (1) can be estimated from a series of laboratory studies conducted in 1970 and 1972 at the Great Lakes Fishery Laboratory (GLFL), Ann Arbor (D. Rottiers, D. O'Conner, and T. Edsall, unpublished data), using lake trout obtained as fingerlings from the Jordan River National Fish Hatchery. Fish of three initial size-classes (about 40, 250, and 1200 g) were held in tanks at 12°C, and fish of the smallest size-class were held at eight temperatures (3.5, 5.0, 7.0, 10.0, 12.0, 15.0, 18.0, and 20.0°C). All fish for a particular experimental condition were grouped in a single tank. All fish were fed excess rations of freshly thawed pieces of alewife (*Alosa pseudoharengus*) twice daily throughout the 12-wk experiment, with the exception of one 2-wk period. During this 2-wk period, insufficient supplies of alewife made it necessary to feed some groups of fish (at 5, 7, 10, 15, and 18°C in 1970) with dry fish pellets. All data from this exceptional 2-wk interval were omitted. Uneaten food was recovered and its weight subtracted from the original amount fed to determine the daily food consumption.

Weight dependence of C_{max} was evaluated using the three size-groups studied at 12°C. In these tanks, consumption and growth of the fish declined noticeably in the latter part of the experiment, suggesting that the fish were stressed in some way. Estimates of maximum consumption were made using the four highest values for each group. This approach is comparable to that used by Brett (1971a) for estimating C_{max} of sockeye salmon, *Oncorhynchus nerka*.

The natural logarithm of the weekly average of daily observations for C_{max} ($g \cdot g^{-1} \cdot d^{-1}$) for each tank of fish was regressed against the natural logarithm of average individual fish weight for the tank. The resulting regression equation for fish at 12°C:

$$\text{(2)} \quad \log_e C_{max} = -1.503 - 0.307 \log_e W,$$

fit the data closely ($r^2 = 0.99$). The weight dependence coefficient, $b = -0.307$, falls between Elliott's (1976b) estimates of -0.241 for brown trout, *Salmo trutta*, at 12°C and the value -0.35 which was estimated for sockeye salmon at 15°C (analysis of data in Brett 1971a). It is within the range (-0.302 to -0.335) found by Niimi and Beamish (1973) for largemouth bass, *Micropterus salmoides*.

The temperature dependence of C_{max} was evaluated for temperatures up to and including 10°C, because this is the range of temperatures typically occupied by lake trout in Lake Michigan. The weekly average of daily C_{max} observations for each tank was adjusted to equivalent consumption for a 50-g fish using the weight dependence exponent, -0.307. The natural logarithms of these values were regressed on average weekly temperature for each tank. The following regression equation provided a good fit ($r^2 = 0.78$, $P < 0.001$, $n = 75$, 50-g fish).

$$\text{(3)} \quad \log_e C_{max} = -4.033 + 0.123T$$

Residuals from this regression and the previous one were evenly scattered about the regression lines, indicating homogeneous variance for both regressions.

Solving each of the two regression equations given above for C_{max} at 12°C and 50 g revealed that the equation for temperature dependence (3) gives an estimate 15% higher than that from the weight dependence equation (2). Because the weight dependence relationship was derived from a relatively small data set ($n = 12$), we chose to use the weight dependence slope in combination with the intercept and slope from the temperature dependence function. The intercept in $g \cdot g^{-1} \cdot d^{-1}$ for the combined functions will be:

$$a = (e^{-4.033}/50^{-0.307}) = 0.059.$$

The complete model for C_{max} in $g \cdot g^{-1} \cdot d^{-1}$ is therefore:

$$\text{(4)} \quad C_{max} = 0.059\, W^{-0.307} \cdot e^{0.123T}.$$

The intercept should be considered a conservative first approximation because the twice-per-day feeding schedule may not have been sufficient for the trout to attain their absolute upper limit. We chose not to use multiple regression for analysis of the consumption data because the experimental design was not fully crossed.

STANDARD AND ACTIVE METABOLISM

That part of a fish's consumed energy that is used to assimilate the remainder is called the heat increment (Niimi and Beamish 1973) or the specific dynamic action, *SDA*. Beamish (1974) found *SDA* to be 17% of assimilated or usable consumption ($C-F$) for fish on a piscivorous diet, and this proportion was relatively independent of temperature and ration size. Kitchell et al. (1977) modeled *SDA* as a (smaller) proportion of total consumption; this may lead to small errors when diet shifts from invertebrates to fish or a mixture. Here we assume *SDA* is a constant proportion of $C-F$:

$$SDA = 0.17 \cdot (C-F).$$

A general model for total daily metabolism, R_U in $g\ O_2 \cdot d^{-1}$ (5), of a salmonid (excluding *SDA*) was obtained by merging functions for weight and temperature dependence of standard metabolism with that for swimming speed dependence of metabolism (Stewart 1980):

$$\text{(5)} \quad R_U = \alpha w^{\beta} \cdot e^{\rho T} \cdot e^{\nu U}$$

where, U = swimming speed, $cm \cdot s^{-1}$
and, α, β, ρ, ν = empirical constants.

An extensive study of lake trout metabolism as a function of weight, temperature, and swimming speed has been conducted at the GLFL using a flow-through tunnel respirometer (D. V. Rottiers, unpublished data; Bell and Terhume 1970). Fingerlings from the Jordan River National Fish Hatchery were reared to appropriate sizes at GLFL. A total of 578 observations (Table 1) were available for lake trout ranging from 20 to 1800 g, and at temperatures of 3.5, 5, 7, 10, and 15°C. The metabolic rates of fish were measured at swimming speeds of 0.6 to 3.0 body lengths $(bl) \cdot s^{-1}$. Individual fish were used in most experiments although small fish were run in groups of three and very small fish in groups of 20, to provide measurable metabolic rates. Additional observations at 18°C were omitted because they appeared to be above the range of constant exponential increase of metabolism with temperature.

Lake trout were fasted and exercised ($10-15\ cm \cdot s^{-1}$) for 48 h then weighed and moved to the respirometer where they were forced to swim. Oxygen consumption was measured continuously during the 24 h the fish were in the respirometer. During the last 8 h of each test the swimming speed was increased in stepwise fashion ($10\ cm \cdot s^{-1}$ at 90-min intervals) from the initial speed of 10 or $20\ cm \cdot s^{-1}$ to the final speed of 50 or $60\ cm \cdot s^{-1}$. The O_2 level in the respirometer was maintained above 70% saturation (by regulating exchange rate) so that rate of consumption by the fish was reasonably independent of oxygen level (based on analysis of data in Gibson and Fry 1954).

Oxygen was measured using a YSI model 54 oxygen meter that was calibrated daily using the Alsterberg modification of

TABLE 1. Number of observations for metabolism of lake trout, *Salvelinus namaycush* (at various fish weights, swimming speeds and temperatures), that were used for multiple regression analysis (D. V. Rottiers, unpublished data).

Weight range (g)	Swimming speed ($cm \cdot s^{-1}$)	Temperature (°C)					
		3.5	5	7	10	15	Totals
1–200							
	10	0	0	0	0	1	1
	20	7	0	6	13	13	39
	30	7	0	6	13	14	40
	40	7	0	6	9	14	36
	50	7	0	6	3	13	29
	60	0	0	4	3	8	15
201–400							
	20	2	0	5	8	3	18
	30	3	0	9	8	5	25
	40	3	0	9	8	5	25
	50	3	0	9	8	4	24
	60	2	0	5	5	2	14
401–600							
	30	5	1	3	9	11	29
	40	5	1	3	9	11	29
	50	5	1	3	9	11	29
	60	3	0	3	8	7	21
601–800							
	30	7	4	3	4	8	26
	40	7	4	3	4	8	26
	50	7	4	3	4	6	24
	60	6	4	3	3	5	21
801–1000							
	30	4	2	2	8	3	19
	40	4	2	2	8	3	19
	50	4	2	2	8	3	19
	60	3	1	2	8	2	16
1001–2000							
	30	0	2	0	1	3	6
	40	0	3	0	3	4	10
	50	0	3	0	3	4	10
	60	0	2	0	2	4	8
Totals		101	36	97	169	175	578

the Winkler method. Background oxygen consumption rarely exceeded 0.1 $mg \cdot L^{-1}$ and was checked weekly by running the apparatus without fish. Data were corrected whenever background level exceeded 0.1 $mg \cdot L^{-1}$. Flow rates were controlled by varying pump speed.

The metabolism data were analyzed using multiple linear regression to estimate the parameters α, β, ρ, and ν. The least squares solution for the general model presented above (R_U in g $O_2 \cdot d^{-1}$, linearized by $\log_e$ transformation) was:

$$(6) \quad \log_e R_U = \log_e 0.00463 + 0.705 \log_e W + 0.059T + 0.0232U.$$

This model fit the data very well ($R^2 = 0.94$, $P < 0.001$). Examination of residual plots indicated homogeneous variance for each of the predictor variables.

The value 13560 $J \cdot g^{-1}$ O_2 (3240 $cal \cdot g^{-1}$ O_2 or 4.63 $cal \cdot mL^{-1}$ O_2) is an appropriate oxycalorific coefficient for converting oxygen respired to energy utilized for a salmonid (Elliott and Davidson 1975; Elliott 1976b). For lake trout with 6280 $J \cdot g^{-1}$ (1500 $cal \cdot g^{-1}$) wet weight, the model for metabolism, R_U (6), can be converted to weight-specific metabolism by changing β to $\beta - 1$ and correcting the intercept, $\alpha = 0.00463$, for relative energy density of oxygen and fish. Thus,

$$\alpha = 0.00463\ (13560/6280) = 0.0100,$$

and the complete model for R_U in terms of gram equivalents (or $g \cdot g^{-1} \cdot d^{-1}$) becomes:

$$R_U = 0.0100\ W^{-0.295} \cdot e^{0.059T} \cdot e^{0.0232U}.$$

In the context of the growth model developed below, energy density, Q, of both the predator and its principal forage may vary seasonally and ontogenetically; α is therefore variable. We begin each simulation with $\alpha = 0.00463$. This value is corrected each day for the relative Q's of oxygen and the food being eaten. Daily specific metabolism is then computed in terms of gram equivalents of the day's forage and can be entered into the mass-balance equation along with daily specific consumption and other components of the energy budget (all expressed in food equivalents per gram fish weight per day). Daily specific growth rate for the average individual fish is therefore computed initially in terms of gram food equivalents per gram body weight per day. To estimate the total growth increment for a given time step, we corrected the growth estimate in food terms for relative Q's of food and fish (see Model Development).

Smith et al. (1978) estimated temperature dependence of routine metabolism of juvenile lake trout (1–4 g) by direct calorimetry. The fish were placed in groups in the metabolic chamber, a modified bomb calorimeter, and changes in water temperature were measured. Results from experiments done at 6, 10, 15, and 18°C indicate exponentially increasing metabolism between 6 and 15°C with a leveling off of the rate of increase between 15 and 18°C. This pattern is in good agreement with our results, suggesting that perhaps Smith et al. were wrong to include the 18°C data in their regression. Assuming a mean weight of 2 g and a weight dependence coefficient of $\beta = 0.705$, least-squares regression estimate of daily metabolism ($g\ O_2 \cdot d^{-1}$) for a 1-g fish versus temperature over the range 6–15°C was $0.0053\ e^{0.0635T}$ ($r^2 = 0.99$, $n = 3$, our analysis of data in Smith et al.). Values for both intercept, $\alpha = 0.0053$, and temperature dependence coefficient, $\rho = 0.063$, are higher than estimates derived above, but not markedly so. Since activity was not controlled or measured in experiments by Smith et al., a relatively high value for α was to be expected. It is to some extent reassuring that results from the two studies are as close as they are, considering the radically different approaches used.

SWIMMING SPEED

To complete the model for metabolism, we needed an estimate of in situ typical daily swimming speed. Optimum swimming speed, U_{OPT} in $cm \cdot s^{-1}$ (7), for salmonids may vary as a positive power function of weight, and temperature dependence of U_{OPT} over low temperatures might be represented as an exponential function for a first approximation (Ware 1978; Stewart 1980).

$$(7) \qquad U_{OPT} = \omega W^{\delta} \cdot e^{\phi T}$$

where, ω, δ, ϕ = empirical constants.

There are no lake trout data available from which to estimate the temperature dependence coefficient, ϕ. As a working hypothesis, this parameter is set to 0.0405 ($Q_{10} = 1.5$), the value estimated by Stewart (1980) from published laboratory observations on temperature dependence of maximum swimming capacity in coho, *Oncorhynchus kisutch* (e.g. Griffiths and Alderdice 1972).

Ware (1978) used hydrodynamics theory combined with considerations of visual acuity, food size, and concentration to estimate optimum foraging and cruising speeds for sockeye salmon. Weight dependence of swimming speed was about 0.13 for both optima. Given the probable marked difference between the typical daily swimming behavior of planktivorous sockeye and epibenthic, piscivorous lake trout, we assumed that lake trout would have a lower weight dependence coefficient. We used $\delta = 0.05$ as a first hypothesis. (If Ware's value of 0.13 is used with a corresponding appropriate value for ω, consumption estimates for the average individual would be about 2% lower over 9 yr of life in the lake.)

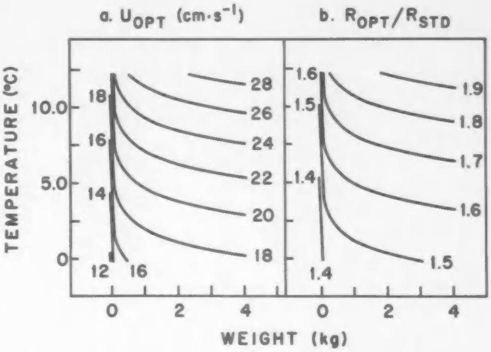

FIG. 1. Contour plots for (a) relationship for theoretical speed (U_{OPT}, $cm \cdot s^{-1}$) of lake trout to weight and temperature; and (b) relationship of the ratio R_{OPT}/R_{STD} to weight and temperature for lake trout.

Large lake trout have been tracked using ultrasonic tags in Lake Superior during the spawning season (R. Horrall, Laboratory of Limnology, University of Wisconsin, Madison, WI, personal communication). Six of the tagged fish were observed to swim; little or no movement was observed for others after tagging, indicating that either the tags fell off or the fish remained stationary. Analysis of tracks showing movement yielded an estimate of $0.46\ bl \cdot s^{-1}$ or $36\ cm \cdot s^{-1}$ for fish averaging 77.5 cm total length. Fish of 77.5 cm should weigh 5220 g based on a length-weight equation for lake trout from central Lake Michigan (Rybicki and Keller 1978). The tracked fish had been displaced from their spawning reef, and thus may have moved faster than would be the case at other times of the year. As a working hypothesis, the swimming speed estimate was arbitrarily reduced 25% to $27\ cm \cdot s^{-1}$ to compensate for this possible bias.

Using $27\ cm \cdot s^{-1}$ at 5220 g with $\delta = 0.05$ gives:

$$U_{OPT} = 17.6W^{0.05}$$

Adding the temperature dependence term and correcting $17.6\ cm \cdot s^{-1}$ to 11.7 at 0°C gives the following completed model for typical in situ swimming speed of lake trout in $cm \cdot s^{-1}$:

$$(8) \qquad U_{OPT} = 11.7\ W^{0.05} \cdot e^{0.0405T}.$$

Total metabolism at typical in situ swimming speed is defined as:

$$R_{OPT} = \alpha W^{\beta} \cdot e^{\rho T} \cdot e^{\nu U_{OPT}}.$$

Equation (8) indicates swimming speeds will range from $11.7\ cm \cdot s^{-1}$ for 1-g fish at 0°C to about $28\ cm \cdot s^{-1}$ at 12°C for the largest lake trout found in Lake Michigan (Fig. 1a).

The increment in metabolism above standard metabolism (R_{STD}) due to activity, R_{OPT}/R_{STD} or $e^{\nu U_{OPT}}$, ranges from 1.4 at low temperature and small size to 1.9 at high temperature and large size (Fig. 1b). R_{OPT}/R_{STD} may be two times higher for large rainbow than for lake trout; large sockeye may be up to three times higher than lake trout (Stewart 1980). This may be reasonable considering the relatively pelagic lifestyle of large rainbow and sockeye (Brett 1971b); lake trout on the other hand are more typically found on or near the bottom.

EXCRETION

Proportion of consumption excreted, E/C, may vary as a function of temperature and ration (Elliott 1976a):

$$E/C = \epsilon_1 T^{\epsilon_2} \cdot e^{\epsilon_3 P}$$

where, P = ration, C_{max}/C,
and $\epsilon_1, \epsilon_2, \epsilon_3$ = empirical constants.

Parameter values reported by Elliott for brown trout eating amphipods were $\epsilon_1 = 0.0252$, $\epsilon_2 = 0.58$, and $\epsilon_3 = -0.299$. Proportion excreted therefore increases with increasing temperature and decreases with increasing ration. Since egested calories cannot be excreted, we modified the value for ϵ_1 to give an expression for egestion that was independent of fraction of ingested food that is indigestible. At the average values of 10°C and a ration of $P = 0.5$, $F/C = 0.174$ from Elliott's equation, and proportion of food assimilated is $(C-F)/C = 0.826$. An approximate corrected value for ϵ_1 is $0.0259/0.826 = 0.0314$, and the revised equation for excretion of assimilated food is

$$E/(C-F) = 0.0314 T^{0.58} \cdot e^{-0.299P}. \tag{9}$$

Daily specific excretion in grams food per gram body weight per day is obtained by multiplying both sides of the equation by $C-F$.

EGESTION: A GENERAL MODEL FOR MIXED DIETS

Proportion of consumption egested, F/C, may also vary as a function of temperature and ration (Brockson and Bugge 1974; Elliott 1976a; Caulton 1978). Elliott proposed

$$F/C = f_1 T^{f_2} \cdot e^{f_3 P} \tag{10}$$

where, f_1, f_2, f_3 = empirical constants.

Elliott's parameter estimates for an invertebrate diet were $f_1 = 0.212$, $f_2 = -0.222$, and $f_3 = 0.631$. In contrast to excretion, proportion of consumption egested decreases (or assimilation efficiency increases) with increasing temperature, and F/C increases with increasing ration. Unfortunately, a study comparable to that of Elliott (1976a) has never been done for a piscivorous fish or for one on a mixed fish−invertebrate diet.

Weininger (1978) adjusted Elliott's parameter values to obtain a first approximation of a model for egestion when eating only fish. This was done by lowering the value of the intercept, f_1, by a proportion assumed to be indigestible chitin, then increasing the ration dependence coefficient, f_3. These adjustments retain the general shape of the response surface defined by Elliott's model. Weininger's modification gave precise estimates of proportion egested at $P = 0$ and at $P = 1$ (the points used to revise f_3), but gave progressively larger errors as P approaches 0.5. Rather than develop this model for the general case of a mixed fish−invertebrate diet, it was abandoned and the following model implemented. This new model retains the essence of Elliott's model (10, 11) as well as Weininger's modification of it.

The new model starts with F/C for an all invertebrate diet as computed directly from Elliott's (1976a) model and parameter estimates:

$$F/C = 0.212 T^{-0.222} \cdot e^{0.631P}. \tag{11}$$

This estimate is then adjusted for proportion of fish in the diet. In the case of an all invertebrate diet, the adjustments all cancel out and leave Elliott's model.

It is assumed that for any food type there is some minimal proportion that will not be assimilated even under the extremes of very low ration and high temperature. From Elliott's model, a brown trout at 25°C and on near-zero ration of amphipods will still egest just over 10% of the food consumed. The absolutely indigestible proportion of an invertebrate diet, K_{INV}, is therefore assumed to be 0.10. Among the lowest estimates for egestion of a piscivorous diet are Kelso's (1972) estimates for walleye (*Stizostedion v. vitreum*) of 3.1% on a yellow perch (*Perca flavescens*) diet, and 2.1% on an emerald shiner (*Notropis atherinoides*) diet. Values of 5−10% are not unusual, but may not be minimal; the indigestible proportion of a fish diet was arbitrarily set at $K_{FISH} = 0.033$.

The first step in adjusting F/C for fish in the diet is to compute the proportion of the diet that is indigestible, K_1:

$$K_1 = (P_{INV} \cdot K_{INV}) + [(1 - P_{INV}) \cdot K_{FISH}]$$

where P_{INV} = proportion of the day's diet composed of invertebrates: P_{INV} values were read from an input array of diet composition.

The second step is to subtract the indigestible proportion (~chitin) of an all invertebrate diet from the initial F/C estimate; $(F/C) - K_{INV}$ gives an estimate of the proportion of consumption scheduled for egestion that is composed of flesh or other potentially assimilatable materials. When fish are eaten, Elliott's model for F/C will underestimate the value of $(F/C) - K_{INV}$. This must be increased in proportion to the ratio of actual proportion of the diet available for assimilation $(1 - K_1)$ and that proportion potentially assimilatable from an all invertebrate diet $(1 - K_{INV})$. Finally, this adjusted value for potentially assimilatable materials that are scheduled for egestion is added to the proportion of the diet that is indigestible, K_1, to yield an estimate of proportion of consumption to be egested for the particular mix of fish and invertebrates in the diet, F_{ADJ}/C. The complete general model for mixed diets is therefore:

$$F/C = f_1 T^{f_2} \cdot e^{f_3 P}$$

$$K_1 = (P_{INV} \cdot K_{INV}) + [(1 - P_{INV}) \cdot K_{FISH}] \tag{12}$$

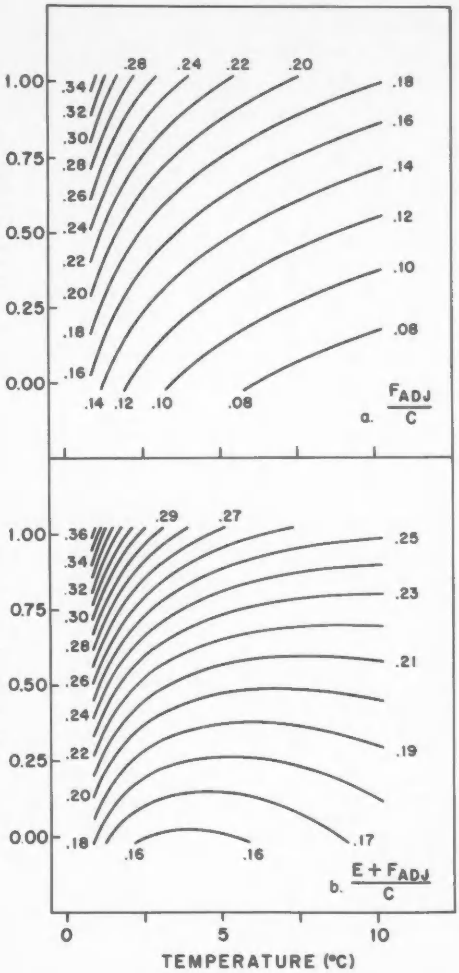

FIG. 2. (a) Contour plot (200-g lake trout eating all fish) for relationship of proportion of consumed food that is egested (F_{ADJ}/C) to temperature and ration ($P = C/C_{MAX}$); and (b) contour plot (for a similar lake trout) for relationship of total waste loss, $(E + F_{ADJ})/C$, to temperature and ration.

$$F_{ADJ}/C = \left[\left(\frac{F}{C} - K_{INV}\right) \cdot \frac{1 - K_I}{1 - K_{INV}}\right] + K_I.$$

Daily specific egestion in grams food per gram body weight per day is then obtained by multiplying F_{ADJ}/C by C. It can be easily demonstrated that this model reduces to the original F/C when $P_{INV} = 1$, in which case $K_I = K_{INV}$.

Equation (12) estimates egestion for lake trout on an all fish diet ranging from about 0.08 at 10°C and low rations to 0.34 at low temperatures and maximum ration (Fig. 2a). At midsummer conditions (10°C and $P = 0.5$), 12% of consumption is egested. Adding F_{ADJ}/C (12) to $E/(C - F)$ (9) gives estimates of total proportion of consumption going to waste losses of 0.16–0.36 (Fig. 2b).

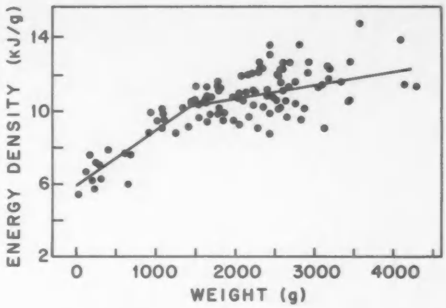

FIG. 3. Weight dependence of energy density ($J \cdot g^{-1}$ wet weight) for lake trout from Lake Michigan (based on Rottiers and Tucker 1982).

ENERGY DENSITY

Knowing the relative energy density or Q's of a predator and its prey is essential if an accurate estimate of predatory consumption is to be made from observed growth rate of the predator. Energy density of 113 individual lake trout from Lake Michigan (collected 1969–71) was determined by bomb calorimetry (Rottiers and Tucker 1982). The Q of lake trout increased with weight, especially for smaller fish (Fig. 3). Attempts to find a simple transformation that would linearize this relationship were unsuccessful. Rather than develop a nonlinear regression model to fit these data, two separate linear regressions were done for fish above and below 1500 g. Using linear equations here makes the growth model developed below mathematically tractable. The regression equations intersect at 1472 g; this is taken as the point to change from one regression line to the next. The model for Q of lake trout in $J \cdot g^{-1}$ wet weight is:

$$(13) \quad Q\ (\leq 1472\ g) = 5700 + 3.08W;$$

$$(14) \quad Q\ (>1472\ g) = 9090 + 0.778W.$$

It is obvious that Q can more than double over the life span of a lake trout (Fig. 3). The smallest observed lake trout have a Q of about 5256 $J \cdot g^{-1}$ (1256 $cal \cdot g^{-1}$) which is higher than the value of 4185 $J \cdot g^{-1}$ (1000 $cal \cdot g^{-1}$) suggested by Winberg (1956) as typical for fishes. The importance of accounting for changes in Q is clear; large, old trout must accumulate twice as much energy as young trout to gain the same amount of weight. Energy density of lake trout may also have a seasonal cycle, but available data were too variable to resolve this with certainty. A seasonal cycle of Q was thus not modeled for the trout.

A Growth Model with Ontogenetic and Seasonal Changes in Energy per Unit Weight

MODEL DEVELOPMENT

Earlier studies have assumed that consumers and their food have the same Q (e.g. Kitchell et al. 1974, 1977; Weininger 1978). The previous section shows that this simple assumption might yield substantial errors when working with salmonids which store large quantities of fat and thereby double their Q over their life. There are also marked differences in Q of invertebrate and fish forage (Cummins and Wuycheck 1971). Furthermore, certain fishes such as the alewife may go through rather dramatic seasonal cycles of Q (Yeo 1978). Consideration of changes in Q should provide a more rigorous estimate of true food needs or predatory impact by lake trout. Solving the energy balance for growth (15) gives an initial estimate for growth of the average individual, ΔB_{IND}, in grams food per gram body weight per day:

$$(15)\quad \Delta B_{\mathrm{IND}} = C - (R_{\mathrm{OPT}} + SDA + F_{\mathrm{ADJ}} + E).$$

In previous studies, growth was added to weight at a given time step (t) to get weight at the next time step ($t + 1$):

$$W_{t+1} = W_t + (\Delta B_{\mathrm{IND}} \cdot W_t).$$

If a predator and its prey differ in energy density, then correction for relative energy value of the predator Q_α, and average energy value of all items in its diet, Q_{FOOD}, had to be made:

$$(16)\quad W_{t+1} = [(W_t \cdot Q_\alpha) + (\Delta B_{\mathrm{IND}} \cdot W_t \cdot Q_{\mathrm{FOOD}})]/Q_\alpha.$$

For the simulations presented below, an array of diet composition for each cohort was input to the model. This included estimates of proportion by weight of four different forage types: invertebrates, (P_{INV}), young-of-the-year alewife, (P_{YOY}), adult alewife, (P_{ADU}), and other fish, (P_{OTH}). Since these proportions may change seasonally, diet composition is reevaluated at each time step (see following section, Food Habits of Lake Trout). Energy density of invertebrates, Q_{INV}, and other fish, Q_{OTH}, were treated as constants. Energy density of alewife is known to follow a seasonal cycle. Observed values at four different times of the year were used to compute a value for energy density of alewife, Q_{ALE}, on each day of the year by linear interpolation. From proportional diet composition and observed Q's of forage, the average energy density of the predator's diet, Q_{FOOD}, can be calculated.

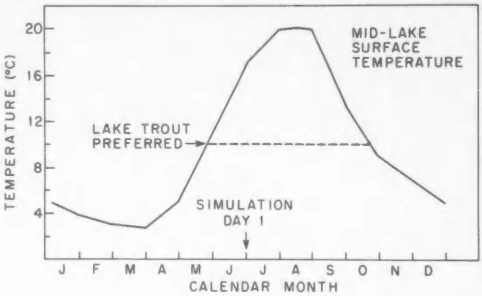

FIG. 4. Seasonal cycle of midlake surface water temperature in Lake Michigan based on Ayers (1962), and temperature (10°C, dashed line) assumed to be occupied by lake trout when surface water gets warmer than 10°C during summer months.

$$Q_{\mathrm{FOOD}} = (P_{\mathrm{INV}} \cdot Q_{\mathrm{INV}}) + (P_{\mathrm{OTH}} \cdot Q_{\mathrm{OTH}}) + [(P_{\mathrm{YOY}} + P_{\mathrm{ADU}}) \cdot Q_{\mathrm{ALE}}].$$

To simulate weight change in a fish exhibiting seasonal cycles in energy (but not ontogenetic changes), we found the energy value of the consumer for the next time step, $Q_{\alpha 2}$, using the interpolation scheme. The weight change equation is as eq. (16), with the divisor replaced by $Q_{\alpha 2}$.

Calculation of growth for a fish with marked ontogenetic changes in energy density is readily resolvable for the case when energy density is a linear function of body weight. Replacing Q_α in eq. (16) with $Q_\alpha + Q_\beta \cdot W$ gives:

$$(17)\quad W_{t+1} = [W_t \cdot (Q_\alpha + Q_\beta \cdot W_t) + (\Delta B_{\mathrm{IND}} \cdot W_t \cdot Q_{\mathrm{FOOD}})]/(Q_\alpha + Q_\beta \cdot W_{t+1}).$$

This can be solved for W_{t+1} using the quadratic formula.

$$(18)\quad W_{t+1} = \frac{-Q_\alpha + \sqrt{Q_\alpha^2 + [4 \cdot Q_\beta \cdot (W_t \cdot (Q_\alpha + Q_\beta \cdot W_t) + (\Delta B_{\mathrm{IND}} \cdot W_t \cdot Q_{\mathrm{FOOD}}))]}}{2 \cdot Q_\beta}.$$

Finally, growth of a predator with both a seasonal cycle and an ontogenetic change of energy density can be computed. In this case, the value of Q_α at time t will be changing to $Q_{\alpha 2}$ at $t + 1$; $Q_{\alpha 2}$ comes from the interpolation scheme described above. W_{t+1} is found by solving:

$$(19)\quad Q_\beta \cdot W^2_{t+1} + Q_{\alpha 2} \cdot W_{t+1} - [W_t \cdot (Q_\alpha + Q_\beta \cdot W_t) + (\Delta B_{\mathrm{IND}} \cdot W_t \cdot Q_{\mathrm{FOOD}})] = 0.$$

When growth of an individual, ΔB_{IND} (15), has been adjusted for changes in Q using eq. 16, 17, 18, or 19, it will be referred to as adjusted growth, ΔB_{ADJ}.

The Lake Michigan Lake Trout Population: Site Specific Variables

WATER TEMPERATURE AND PREFERRED TEMPERATURE

Actual temperature occupied by the typical individual of a fish population appears to be an interaction of available temperature, preferred temperature, and to some extent, recent temperature experience or acclimation (Neill and Magnuson 1974). It is believed that water temperature of a lake changes at a rate which permits nearly continuous acclimation to whatever temperature the fish occupies, except for the special case of vertical migration through a sharp temperature gradient. For lake trout, we hypothesized that they will occupy the warmest available temperature up to but not exceeding their preferred temperature (Beitinger and Magnuson 1975).

The final temperature preferred by yearling lake trout in laboratory studies was 11.7°C, independent of starting acclimation temperature (McCauley and Tait 1970). Field observations on lake trout suggest they normally occupy a temperature about 2°C lower than the laboratory determined preferendum (Ferguson 1958; Spangler and Berst 1976). Factors such as relative food availability, predator avoidance, interference competition, etc., will modify orientation to the preferred temperature (Magnuson et al. 1979). An observed thermal history is thus more appropriate when known. For the simulations presented herein, the preferred or maximum summer temperature occupied in Lake Michigan is assumed to be 10°C. The annual temperature cycle (Fig. 4) was estimated from midlake data from Ayers (1962), and this agrees with observations of Carr et al. (1973).

FOOD HABITS OF LAKE TROUT

Actual food habits of lake trout (expressed as proportional, wet-weight diet composition) are critical site-specific data needed for quantitative evaluation of predatory impact upon various forage organisms. Gary Eck (GLFL, U.S.F.W.S., Ann Arbor, MI, personal communication) has in progress an extensive study of seasonal food habitats of lake trout in Lake Michigan, and has provided us with preliminary data expressed in percentage occurrence of various food types. We converted these data to approximate proportions by weight using the following assumed typical live weights (g): sculpin adults = 20, juveniles = 2; smelt adults = 32, juveniles = 4; alewife (spring/summer/fall) adults = 34/29/33, juveniles = 3.9/10/3.7. These data were based on values for fish caught in experimental trawls in Lake Michigan (L. Moffett, Michigan Department of Natural Resources, personal communication), and for alewife, based on average lengths of those in the trout stomachs (G. Eck, GLFL, U.S.F.W.S., Ann Arbor, MI, personal communication). Data were combined with observations of Dryer et al. (1965) on food habits of small lake trout in Lake Superior, since Eck caught relatively few small juveniles in Lake Michigan. Dryer et al. indicated that benthic invertebrates may be an important component of the diet in juvenile lake trout. The proportions of invertebrates in the diet of lake trout of various size-classes were estimated from Dryer et al. to be 0.75 (100−200 mm fish length), 0.30 (200−300 mm), 0.10 (300−400 mm), 0.0 (> 400 mm).

The above information was combined as model input (Table 2) of diet composition by weight for various size-classes of lake trout in each of three different seasons — winter and spring (Dec.−May), summer (June−Oct.), and fall (Nov.). The one month fall season is the period when Eck noted that juvenile alewife were moving into deeper water and were preyed upon heavily by lake trout. Data for smelt and sculpin were merged to form a single food class termed "other fish." This gives proportions of four food types for the lake trout: invertebrates (P_{INV}), young-of-the-year alewife (P_{YOY}), adult alewife (P_{ADU}), and other fish (P_{OTH}).

Diet composition of Lake Michigan lake trout changes noticeably with size (Table 2). Small trout feed on invertebrates and small fishes. As size increases to about 1500 g, smelt and sculpin are increasingly important diet components. Trout larger than about 2000 g eat almost entirely adult alewife, except during the fall. The observations of Eck on summer food habits of lake trout are in very close agreement with those of Rybicki and Keller (1978).

The model developed above also requires estimates of Q for the various food types in the diet, both to convert metabolism to grams food equivalent and to convert growth in grams food equivalent to growth in body weight, ΔB_{ADJ}. In the Great Lakes, *Mysis relicta* and *Pontoporeia hoyi* are important components of the invertebrate forage of young lake trout (Dryer et al. 1965). Estimates of wet weight Q for these two invertebrates could not be located; it was assumed that they are typical Malacostraca with 4310 $J \cdot g^{-1}$ wet weight (Cummins and Wuycheck 1971).

Smelt have been studied by Foltz (1974) and data in an appendix of his thesis provide a basis for estimating wet weight Q. Foltz and Norden (1977) only gave dry weight data. Foltz presented seasonal data for both sexes of 3- and 4-yr-old smelt. A seasonal cycle of Q was evident, but not nearly as pronounced as that for alewife (Yeo 1978). The overall average value was estimated to be 5700 $J \cdot g^{-1}$ wet weight based on the 20 mean values (five dates, two sexes, two age-classes) presented by Foltz. This value was used as a constant for "other fish," Q_{OTH}, in the lake trout diet. Brockson et al. (1968) estimated 5210 $J \cdot g^{-1}$ wet weight for *Cottus perplexus*, which is reasonably close to the smelt estimate. Estimates of Q for slimy sculpin (*Cottus cognatus*, 5740 $J \cdot g^{-1}$) and deepwater sculpin (*Myoxocephalus thompsoni*, 5420 $J \cdot g^{-1}$) from Lake Michigan are also closely similar (Rottiers and Tucker 1982).

Alewife show a pronounced seasonal cycle of energy density, Q_{ALE}, and this was modeled using energy estimates for four different times of the year with linear interpolation between the observations. The values for Q_{ALE} (calculated from data presented in Yeo 1978) were 5330 (June), 6450 (Sept.), 9080 (Nov.), and 5270 (March) $J \cdot g^{-1}$. There may also be an ontogenetic change during the first 1 or 2 yr of life but Yeo did not have enough data on small fish to draw definite conclusions. If there is an ontogenetic change for older age-classes, it is obscured by the seasonal cycle. If YOY alewife have relatively lower Q, then simulation estimates of YOY alewife consumption by lake trout will be conservative.

GROWTH

Growth of lake trout is faster in the southern part of Lake

TABLE 2. Proportional composition by weight of four food categories in the diet of Lake Michigan lake trout (*Salvelinus namaycush*) at various times of the year. Day 1 of a modeling simulation is July 1. Based on data from Eck (GLFL, Ann Arbor, personal communication) and Dryer et al. (1965).

Cohort	Simulation days		Food type				Cohort	Simulation days		Food type			
	Start	End	P_{INV}	P_{OTH}	P_{YOY}	P_{ADU}		Start	End	P_{INV}	P_{OTH}	P_{YOY}	P_{ADU}
1	1	120	0.75	0.21	0.04	0.0	4	1	120	0.0	0.17	0.0	0.83
	121	150	0.30	0.66	0.04	0.0		121	150	0.0	0.16	0.84	0.0
	151	330	0.30	0.54	0.16	0.0		151	330	0.0	0.43	0.0	0.57
	331	360	0.30	0.13	0.57	0.0		331	360	0.0	0.17	0.0	0.83
2	1	120	0.10	0.53	0.0	0.37	5	1	120	0.0	0.17	0.0	0.83
	121	150	0.10	0.31	0.59	0.0		121	150	0.0	0.03	0.97	0.0
	151	330	0.10	0.72	0.0	0.18		151	330	0.0	0.23	0.0	0.77
	331	360	0.10	0.53	0.0	0.39		331	360	0.0	0.06	0.0	0.94
3	1	120	0.0	0.53	0.0	0.47	6	1	120	0.0	0.06	0.0	0.94
	121	150	0.0	0.35	0.65	0.0		121	150	0.0	0.03	0.97	0.0
	151	330	0.0	0.57	0.0	0.43		151	330	0.0	0.23	0.0	0.77
	331	360	0.0	0.53	0.0	0.47		331	360	0.0	0.06	0.0	0.94
							7–9	1	120	0.0	0.02	0.0	0.98
								121	150	0.0	0.03	0.97	0.0
								151	330	0.0	0.14	0.0	0.86
								331	360	0.0	0.02	0.0	0.98

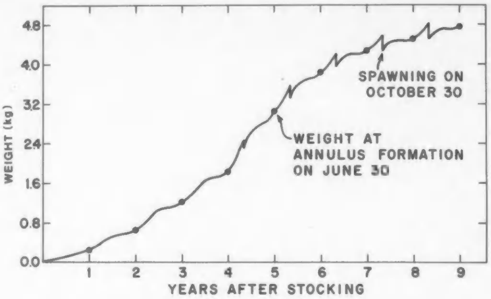

FIG. 5. Simulated growth of the average individual lake trout in Lake Michigan as fit to the annual end points of growth (●) based on the average of values estimated for three different parts of Lake Michigan — south, mid, north — by Rybicki and Keller (1978).

Michigan than in the northern part (Rybicki and Keller 1978). Our modeling simulations are fit to the annual end points of growth. We used the average southern, midlake, and northern observations given by Rybicki and Keller (1978: p. 41). Their mean size estimate for age-class 8 in the southern region was actually lower than for age 7, perhaps due to the small sample size. We therefore used average size of midlake and northern fish for age-classes 8 and 9 (i.e. growth end points for model cohorts 7 and 8). The model was extended to the end of 9 yr in the lake by assuming the weight gain between cohorts 7 and 8, 239 g, was the same as that between 8 and 9. Final weight for age-class 2 (cohort 1) is taken from Weininger (1978) and based on a subset of Rybicki and Keller's original data. The growth curve thus derived was assumed to be representative of a lake-wide average (Fig. 5). The starting weight was set at 20 g on June 30 (age-class 1+), and weight in grams at 1-yr intervals thereafter were: 1 = 260, 2 = 659, 3 = 1216, 4 = 1828, 5 = 3044, 6 = 3842, 7 = 4281, 8 = 4520, 9 = 4760. June 30 corresponds approximately to the time lake trout are stocked as well as the typical time for formation of scale annuli.

Growth depends on P (ration or C/C_{max} defined above) as well as C_{max}. Since P is unknown, it must be estimated by simulation. For a given cohort, P is given an arbitrary intermediate starting value, such as 0.5. A growth simulation is then run and the simulated annual end point of growth is compared to the desired (or observed) annual end point. P is then adjusted up or down depending on whether the simulation was too high or too low, and additional iterations are done until observed final weight is obtained with the desired degree of accuracy (e.g. ± 0.2%). The iteration routine used was that developed by Weininger (1978). The actual value of P may vary seasonally with food availability, but this possibility cannot be tested because we only have annual estimates of weight.

REPRODUCTION

Lake trout in Lake Michigan apparently spawn each fall,

TABLE 3. Symbols and estimated (or postulated) parameter values used to implement an energetics model for Lake Michigan lake trout (*Salvelinus namaycush*).

Process	Symbol	Parameter description	Parameter value
Consumption	a	Intercept for maximum consumption (C_{max}), @ 0°C	0.059 ($g \cdot g^{-1} \cdot d^{-1}$)
	b	Weight dependence coefficient for C_{max}	−0.307
	q	Temperature dependence coefficient for C_{max}	0.123
	T_{PREF}	Preferred temperature (upper bound on C_{max})	10.0°C
Metabolism	α	Intercept for metabolism	0.00463 ($g\ O_2 \cdot d^{-1}$)
	β	Coefficient for metabolism vs. weight	−0.295
	ρ	Coefficient for metabolism vs. temperature	0.059
	ν	Coefficient for metabolism vs. swimming speed	0.0232
Swimming speed	ω	Intercept for optimum swimming speed	11.7 ($cm \cdot s^{-1}$)
	δ	Coefficient for optimum swimming speed vs. weight	0.05
	ϕ	Coefficient for optimum swimming speed vs. temperature	0.0405
	SDA	Specific dynamic action coefficient	0.17
Egestion	f_1	Intercept for proportion of consumed food egested	0.212
	f_2	Coefficient for egestion vs. temperature	−0.222
	f_3	Coefficient for egestion vs. feeding level	0.631
	K_{INV}	Indigestible fraction of invertebrate diet	0.10
	K_{FISH}	Indigestible fraction of fish diet	0.033
Excretion	ϵ_1	Intercept for proportion of consumed food excreted	0.0314
	ϵ_2	Coefficient for excretion vs. temperature	0.58
	ϵ_3	Coefficient for excretion vs. feeding level	−0.299

but for some unknown reason, there has been virtually no recruitment of naturally spawned young. Reproductive products lost each year are nonetheless weight gained through additional consumption and should be considered when evaluating predation and conversion efficiency. Female lake trout weighing less than 5 kg lay an estimated 12.1% body weight as eggs (Eschmeyer 1955: p. 66, assuming 0.1 $g \cdot egg^{-1}$). Male gonads change from an average maximum of 3.1% body weight to 1.7% at end of the spawning season for a loss of 1.4%; thus, average loss for both sexes is 6.8% (Eschmeyer 1955). For simulations presented below, this average constant proportion of body weight was lost as a step function on day 120 (October 30) of cohorts 5−9 (Fig. 5).

NUMBERS, BIOMASS, MORTALITY, AND PRODUCTION

Model components and site specific variables presented above are sufficient to simulate the average individual lake trout in Lake Michigan. Extension to the entire Lake Michigan population requires estimates of numbers stocked and mortality rates. Past, present, and projected future stocking rates for lake trout in Lake Michigan, 1965−90, were summarized in Stewart (1980). To simulate the population in any given year, we estimated numbers starting in each age-class or cohort from actual numbers stocked in that year and up to 8 previous years. Mortality rates presented below were used for determining numbers in all but the first cohort.

Rybicki and Keller (1978) estimated instantaneous total mortality rate for lake trout vulnerable to the sport fishery in Lake Michigan (i.e. after 4 yr in the lake) as a function of fishing effort in various statistical districts. They extrapolated this relationship to zero fishing effort to get an estimate of 24.7% average annual natural mortality, or 0.284 for the average instantaneous natural mortality rate. From the difference between total mortality and natural mortality, Rybicki and Keller estimated the average lake-wide annual fishing mortality to be 30%. The average annual instantaneous harvest rate was therefore $0.3567 \cdot yr^{-1}$, but fishing is mostly concentrated in the 6-mo period May through October (Rybicki and Keller 1978). The annual instantaneous harvest rate is therefore $0.7134 \cdot yr^{-1}$ during May−October and zero for the remaining 6 mo.

For lake trout during their first 4 yr in the lake, we used Rybicki and Keller's (1978) estimate of 37% annual natural mortality rate. This was based on an estimate for age-class 2 from trawling catch-per-unit-effort in only one area of the lake. Given that rate estimates can vary somewhat for different areas of the lake, their estimate for young age-classes needs to be substantiated by further studies. The model presented herein is relatively sensitive to errors in mortality rate estimates.

Total annual survival rates used to determine numbers starting each cohort were derived from the above natural and harvest mortality rates. During the first 4 yr, there was relatively little fishing mortality; the primary fishing method, trolling, seems to select strongly for large fish. Fishing was assumed to become an important source of mortality in the fifth summer (i.e. at start of eleventh month, May, of the fourth cohort).

Rybicki and Keller (1978) assumed population decreases were continuously exponential with natural mortality evenly distributed over the entire year, and fishing evenly spread over May−October. The same model was used herein. Daily instantaneous natural mortality was estimated to be $0.284/360 = 7.889 \times 10^{-4}$; daily instantaneous fishing mortality was $0.7134/360 = 19.817 \times 10^{-4}$ during the 6-mo fishing season. Gross and net production for the time step can be estimated as:

$$PR_{GROSS} = (W_{t+1} - W_t) \cdot N_t,$$

and

$$PR_{\mathrm{NET}} = PR_{\mathrm{GROSS}} - (Z \cdot W_t),$$

where Z is total loss of numbers to mortality during the time step. This completes the equation series for the energetics model, as well as derivation of the various parameters for the Lake Michigan lake trout population. Parameters for the primary energetics functions are summarized in Table 3.

Energetics of the Average Individual Lake Trout

Implementation of New Model Features

The above energetics equations extend Weininger's (1978) original development for lake trout. Changes include: (1) a slightly different growth curve with higher first-year growth and one more year in the lake; (2) a new egestion function for mixed diets to replace Weininger's fish-diet modification of Elliott's (1976a) egestion model; (3) a growth model with ontogenetic changes of Q in trout and seasonal cycle of Q in its prey to replace assumed Q food = Q trout = 4440 $\mathrm{J \cdot g^{-1}}$ as a constant throughout; (4) reproductive energy losses for mature fish compared to none in the earlier version; and (5) a model for size and temperature dependence of swimming speed in place of previously assumed 0.46 $\mathrm{bl \cdot s^{-1}}$ swimming speed constant at $T > 5°\mathrm{C}$, and 0.33 $\mathrm{bl \cdot s^{-1}}$ at $T \leq 5°\mathrm{C}$.

Implementation of these new features revealed that the original version underestimated assimilation efficiency. Gross conversion efficiency of energy (21.8%) over the life of a lake trout, including energy released as gametes, is about twice the conversion efficiency of mass (12.2% herein, or 12.8% estimated by Weininger 1978) because lake trout store large amounts of high-energy fats. Another important conclusion from comparison of results from the two versions is that the model as originally formulated was relatively robust in its estimates of accumulative consumption with an overall increase of only 3.4% between the old and new versions at the end of 8 yr in the lake. The ninth cohort of the new version adds another 18% to the total estimate for the average individual, but relatively few trout survive to this age. This robustness gives added confidence in the model estimate for predatory impact of lake trout on their forage, which was the ultimate objective of this modeling exercise.

The fact that implementation of these model features does not seriously alter the overall consumption estimates obscures the finer-scale changes that have been induced. Consumption, conversion efficiency, and their seasonality have been altered differentially within the different age-classes providing what should be a more realistic model for the life history of lake trout. The new egestion function was not expected to greatly alter either Elliott's model or Weininger's modification for a fish diet, but rather to allow realistic simulation of egestion with mixed diets. The model for linear increase in lake trout energy density implies that large trout consume twice as much food as small trout per unit gain in weight. If Q of the prey has a seasonal cycle, trout will have a parallel seasonal cycle of conversion efficiency. If Q of the prey were lowered a certain percentage, the total consumption estimate would increase by a greater percentage. This emphasizes the importance of modeling energy density of both predator and prey with reasonable accuracy. The assumption that a planktivorous fish has the same Q as zooplankton (e.g. Kitchell et al. 1977, YOY yellow perch), may give a 2- to 3-fold underestimate of zooplankton consumed, since zooplankton typically have 1/2 to 1/3 the Q of a fish. Implementation of the mixed diet means small fish eating invertebrates will have a relatively lower conversion efficiency and need to eat more per unit weight gain than in the original version. Finally, the new swimming speed model increases metabolism for small fish and lowers it for large fish; these two effects compensate each other when integrated over the life of a lake trout.

TABLE 4. Normalized sensitivities of accumulative consumption estimates for Lake Michigan lake trout to deviations of various input parameters (see text for explanation of procedures and normalization).

	Parameter input error			Parameter input error	
Parameter	+10%	−10%	Parameter	+10%	−10%
Consumption			Egestion		
a	−0.05	+0.07	f_1	+0.25	−0.24
b	−0.11	+0.17	f_2	+0.11	−0.10
q	−0.04	+0.05	f_3	+0.08	−0.07
T_{PREF}	+0.28	−0.28	K_{INV}	−0.12	+0.11
Metabolism			K_{FISH}	−0.04	+0.04
α	+0.72	−0.71	Excretion		
β	+1.91	−1.47	ϵ_1	+0.13	−0.12
ρ	+0.32	−0.30	ϵ_2	+0.16	−0.14
ν	+0.40	−0.38	ϵ_3	+0.02	−0.02
Swimming speed			Energy density		
ω	+0.40	−0.38	< 1472 g Q_α	+0.05	−0.05
δ	+0.16	−0.15	< 1472 g Q_β	+0.04	−0.04
ϕ	+0.12	−0.12	> 1472 g Q_α	+0.17	−0.17
SDA	+0.25	−0.24	> 1472 g Q_β	+0.09	−0.09
			Q_{INV}	−0.02	+0.02
			Q_{OTH}	−0.17	+0.18

TABLE 5. Normalized sensitivities of accumulative metabolism (g O_2, $R + SDA$) estimates for Lake Michigan lake trout to deviation of various input parameters (see text for explanation of procedures and normalization).

	Parameter input error			Parameter input error	
Parameter	+10%	−10%	Parameter	+10%	−10%
Consumption			Egestion		
a	0.0	−0.01	f_1	0.0	−0.01
b	0.0	−0.01	f_2	−0.01	0.1
q	+0.02	−0.03	f_3	0.0	−0.01
T_{PREF}	+0.35	−0.38	K_{INV}	0.0	−0.01
Metabolism			K_{FISH}	−0.01	0.0
α	+0.92	−0.92	Excretion		
β	2.45	−1.93	ϵ_1	+0.02	−0.03
ρ	+0.40	−0.39	ϵ_2	+0.03	−0.03
ν	+0.51	−0.50	ϵ_3	0.0	−0.01
Swimming speed			Energy density		
ω	+0.51	−0.50	< 1472 g Q_α	0.0	−0.01
δ	+0.20	−0.20	< 1472 g Q_β	0.0	−0.01
ϕ	+0.15	−0.15	> 1472 g Q_α	+0.03	−0.04
SDA	+0.32	−0.31	> 1472 g Q_β	+0.02	−0.02
			Q_{INV}	−0.01	0.0
			Q_{OTH}	−0.01	0.0

SENSITIVITY ANALYSIS FOR CONSUMPTION AND METABOLISM

A sensitivity analysis was done on the complete energetics model for the average individual lake trout in Lake Michigan using the 9-yr growth curve developed from Rybicki and Keller (1978). The objective was to determine to what extent errors in estimating various model parameters influence estimates of accumulative consumption and metabolism over the life of a lake trout. Each of 26 input parameters was separately varied +10% then −10% and resultant changes in consumption and metabolism were compared to nominal estimates from the standard simulation which used best available parameter estimates derived in preceding sections.

Sensitivity of a computed output value to deviation of an input parameter was calculated using the method presented by Kitchell et al. (1977);

$$S_x(p) = (p \cdot \Delta x)/(x \cdot \Delta p)$$

where, $S_x(p)$ = sensitivity of output parameter x to deviations of input parameter p,
p = nominal value of input parameter p,
Δp = input deviation of input parameter p,
x = nominal value of output parameter x from standard simulation,
and Δx = deviation of output parameter x due to Δp.

A sensitivity of 1.0 indicates that a 10% error in an input parameter will yield a 10% error in estimates of output such as consumption or metabolism. The higher the absolute sensitivity value ($|S_x(p)|$), the greater the potential for error.

Sensitivities to consumption, egestion, excretion, and energy parameters were all quite low (Table 4). Sensitivity to an energy density parameter, however, may be close to 1.0 when the predator is eating only that particular food type. Sensitivity of the consumption estimate to errors in estimating energy content of alewife may be close to 1.0 because it forms a large component of the lake trout's diet. Sensitivity of consumption and metabolism estimates to errors in determining the annual cycle of energy content in alewife were not explicitly evaluated. Highest sensitivities were to the metabolism parameters, α and β, with moderate sensitivities to ρ and ν. Since the swimming speed parameter ω is multiplied by ν, its sensitivity is identical to that of ν; sensitivity is low to other swimming speed parameters. Relative sensitivity of metabolism estimates to input errors of various model parameters (Table 5) follows a pattern almost identical to that of consumption estimates.

Relatively high sensitivity of the various metabolism parameters points to the need for good laboratory studies on metabolism of fishes. In the present model, high sensitivities are offset by good confidence in our parameter estimates which are based on a large data set. More observations of in situ swimming speed would be useful, considering the moderate sensitivity of the parameter ω.

Results of the sensitivity analysis indicate a general robustness of the model, especially for estimating consumption by fitting a known growth curve. This supports conclusions from comparison of old and new versions of this model which were discussed in the previous section. Sensitivities of various parameters appear to be lower for consumption and metabolism estimates than for estimates of growth as presented by Kitchell et al. (1977). The ±10% input errors used here may be reasonable approximations of our confidence in many of the parameters, suggesting that estimates of consumption for the average individual lake trout may be within 20% or less of actual value. Far greater uncertainties are involved in estimates of mortality rates for the entire population and this should be a subject for continuing research. Substantial

TABLE 6. Comparison of weight gain, ration, total consumption, and gross conversion efficiency of biomass for the average individuals of the nine lake trout cohorts in Lake Michigan. Based on an energetics modeling simulation with starting weight of 20 g for the first cohort. Growth curve (Fig. 5) is from Rybicki and Keller (1978). Also given are total weight and energy gained, consumed, and % conversion over 9 yr of life. It was assumed that 6.8% body weight was lost as reproductive products on day 120 (cohorts 5–9 only). Gametes lost were added to weight gained to calculate efficiency; gametes were assumed to have 6240 $J \cdot g^{-1}$ wet weight (Cummins and Wuycheck 1971).

Cohort	Weight (g)	Weight gain (g)	Gametes lost (g)	Ration (C/C_{max})	Food consumed (g)	% Gross conversion efficiency
1	260	240	0	0.749	983	24.4
2	659	399	0	0.562	2062	19.4
3	1216	557	0	0.575	3487	16.0
4	1828	612	0	0.522	4408	13.9
5	3044	1216	167	0.684	8010	17.3
6	3842	798	242	0.546	8160	12.7
7	4281	439	287	0.459	7690	9.4
8	4520	239	311	0.417	7380	7.5
9	4760	240	328	0.420	7710	7.4
Total grams		4740	1335		49890	12.2
Total joules		60.9×10^6	8.3×10^6		317.9×10^6	21.8

deviation from the smooth exponential mortality assumed has obvious important consequences. For example, Kitchell and Breck (1980) demonstrated that lamprey-induced mortality can be strongly pulsed in late summer or early autumn.

CONVERSION EFFICIENCY

Gross conversion efficiency ($\Delta B_{ADJ}/C$) can provide a useful index of the general well-being of a fish under a given set of diet and environmental conditions. Evaluation of conversion over the range of temperatures normally occupied in the field and at growth rates possible at various ration levels can also yield useful information about optimum conditions for a particular species (Webb 1978; Brett and Groves 1979). To evaluate relative well-being of the various cohorts of lake trout in Lake Michigan, we summarized results from the standard simulation for the average individual by cohort to get estimates of overall gross conversion efficiency for each cohort.

Results of this analysis (Table 6) indicated relatively high conversion efficiency for the first cohort and lowest conversion for the oldest two cohorts. Conversion estimates for the older cohorts would appear to be much lower if gamete losses were ignored. Efficiency for the first cohort was unexpectedly high, considering the large invertebrate component in the diet. Cohorts 2–7 show substantial variation, apparently related to the amount of growth occurring in a given year. Fish growing faster are on higher rations and tend to have better conversion.

Higher conversion efficiency at relatively higher rations is displayed in a contour plot of $\Delta B_{ADJ}/C$ versus temperature and daily specific growth rate (Fig. 6). These results with maximum conversion at maximum ration are similar to those of Elliott (1976b) and modeling simulations for yellow perch (Kitchell et al. 1977), but differ from Brett et al.'s (1969) observed maximum conversion at rations slightly below maximum. The model as presently developed consistently

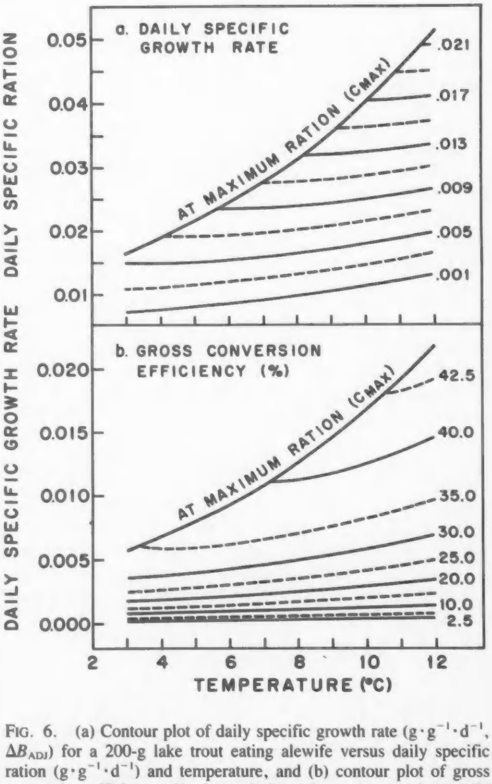

FIG. 6. (a) Contour plot of daily specific growth rate ($g \cdot g^{-1} \cdot d^{-1}$, ΔB_{ADJ}) for a 200-g lake trout eating alewife versus daily specific ration ($g \cdot g^{-1} \cdot d^{-1}$) and temperature, and (b) contour plot of gross conversion efficiency (%) for a similar lake trout at various daily specific growth rates (ΔB_{ADJ}) and temperatures.

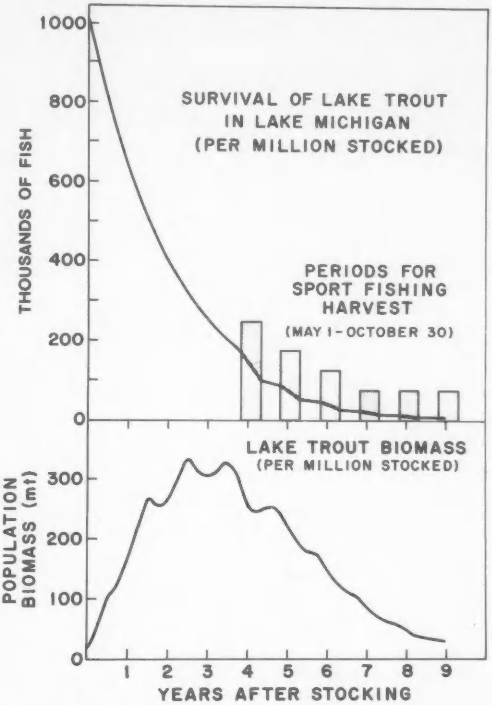

FIG. 7. (*Above*) Survival curve for lake trout in Lake Michigan (per one million smolts stocked) based on natural and fishing mortality coefficients estimated by Rybicki and Keller (1978), and (*below*) buildup and depletion of lake trout biomass per million fish stocked in Lake Michigan based on a modeling simulation using growth rates (Fig. 5) and mortality rates estimated from Rybicki and Keller (1978).

gives results that resemble those derived from carefully controlled experiments by Elliott (1976b).

Predatory Impact of the Lake Trout Population

POPULATION SIMULATION FOR ONE MILLION FISH

Results of the foregoing analyses relate to the average individual lake trout. The full numbers–biomass capabilities of the model were implemented to evaluate (1) build-up of population biomass and consumption over time from stocking to the end of 9 yr, (2) relative importance of different trout cohorts in preying on various forage types, and (3) total predatory impact on alewife by lake trout since they were planted in 1965, with projections of alewife consumption by lake trout to 1990. The mortality coefficients were those derived above from data presented in Rybicki and Keller (1978).

The first two objectives in this section were addressed using modeling simulations for one million lake trout stocked once and followed over 9 yr. This differs from the following simulations of actual populations in the lake in various years since actual numbers in a given cohort of a given year are dependent on the variable numbers stocked in previous years. The population simulation for a million fish indicates a steady build-up of population biomass over the first 3.5 yr to 330 $t \cdot million^{-1}$ stocked (Fig. 7). Biomass may have risen slightly higher, but sport fishing (Fig. 7) becomes important in the fifth summer after stocking (Rybicki and Keller 1978) and this harvesting sharply curtails further increment of total population biomass. As might be expected, total consumption also rises to a peak in the third cohort then declines steadily with older cohorts (Table 7). Because of these time-lags in the system, manipulation of stocking densities for lake trout is not an effective means of moderating fluctuations in prey densities (Stewart et al. 1981). It seems possible, however, that lake trout could partially dampen prey fluctuations by short-term changes in consumption rates, and this would show up as annual variation in growth rate.

A comparison of relative predatory impact of various cohorts on different forage types (Table 7) indicates that invertebrates are most important for the first cohort, while YOY alewife, other fish (smelt and sculpin), and invertebrates are all relatively important in the second and third years in the lake. Older cohorts eat predominately adult alewife as noted earlier (Table 2). A comparison of percent composition of the total diet integrated over all cohorts, indicates a relatively higher importance of invertebrates and other fish in the population simulation compared to the average individual (Table 7, bottom). This of course is related to the large numbers of young trout that do not survive to attack adult alewife in the simulation for the entire population.

POPULATION SIMULATIONS FOR 1965–90

The general concensus over the past 15 yr has been that there is an abundance of forage fishes in Lake Michigan and substantial increases in stocking densities of predatory salmonids are still possible (e.g. Borgeson 1977). This lake trout modeling study was part of a larger effort to evaluate the null hypothesis that salmonids in Lake Michigan are not seriously impacting their forage base (Stewart et al. 1981). As noted above, the model appears to be robust and reasonable confidence can be put in consumption estimates, at least for the average individual. The numbers in each cohort during each year for the period 1965–90 were estimated from actual or projected numbers stocked as summarized in Stewart (1980) and mortality coefficients derived from Rybicki and Keller (1978).

Predation of lake trout on alewife has been building up steadily since 1965 to about 8.4 million $kg \cdot yr^{-1}$ (8400 $t \cdot yr^{-1}$), and is projected to rise to almost 12 000 $t \cdot yr^{-1}$ by 1990 (Fig. 8). This amount of consumption by itself might represent only a small fraction of the alewife biomass in Lake Michigan in a given year as estimated by Hatch et al. (1981). Lake trout, however, represent only about 20% of the predatory salmonids stocked in the lake, and together, the various salmonids could be eating as much as 20–33% of the annual alewife production in some years (Stewart et al. 1981).

Additional simulations were done for 1980–90 with

TABLE 7. Comparison of predatory impact of various lake trout cohorts upon different forage types in Lake Michigan. Based on energetics modeling simulations using the growth curve (Fig. 5) from Rybicki and Keller (1978), and food data from Table 2. All values are for a 360-d simulation year.

	Consumption by average individual[a]				Consumption per million stocked[b]				
Cohort	Invert. (g)	Other fish (g)	YOY alewife (g)	Adult alewife (g)	Invert. (t)	Other fish (t)	YOY alewife (t)	Adult alewife (t)	All foods (t)
1	410	377	197	0	332	289	137	0	758
2	206	1205	111	539	104	602	59	277	1042
3	0	1845	204	1438	0	590	68	462	1120
4	0	1166	332	2907	0	227	70	589	886
5	0	1328	683	6006	0	136	68	608	812
6	0	960	695	6500	0	48	36	354	438
7	0	480	653	6560	0	13	18	188	219
8	0	459	626	6290	0	6	9	96	111
9	0	479	655	6580	0	4	5	52	61
Totals	616	8299	4156	36820	436	1915	470	2626	5447
% of diet	1.2	16.6	8.3	73.8	8.0	35.2	8.6	48.2	100.0

[a] Based on a simulation for a single fish (no mortality).

[b] Based on a simulation for the entire population with mortality functions implemented.

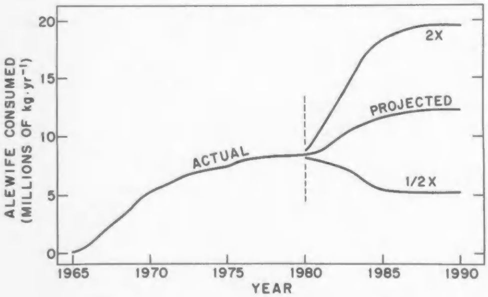

FIG. 8. Estimated actual annual consumption of alewife by lake trout in Lake Michigan during various years based on modeling simulations for the entire trout populations, with projections to 1990. Upper and lower curves between 1980 and 1990 are based on the assumptions that the stocking rate was either held constant at 2× or ½× the 1980 rate.

double and half the number of lake trout stocked in 1980 to determine how rapidly a change in stocking density might be reflected in altered predation rates (Fig. 8). These results emphasize the point made earlier that about 4 yr may be needed to effect a marked change. Reductions in lake trout population biomass can perhaps be effected more rapidly through increased fishing effort. It could be desirable to do this in the event of a collapse of the forage fish populations, but changes of stocking densities for the various salmon would yield quicker responses (Stewart et al. 1981).

The simulation results presented herein were intended to provide first indications of the forage requirements of past, present, and projected future lake trout populations in Lake Michigan. We believe that our overall estimates are reasonable but recognize that a number of refinements should be possible, including evaluation of year-to-year and regional variation of feeding rates and diet composition. As our knowledge of the Great Lakes increases, we anticipate that the modeling approach developed herein will become a progressively more powerful research and management tool.

Summary

1) Analyses indicate that the model developed herein is especially well suited for providing estimates of consumption by fitting a known growth curve. The energetics modeling approach can provide estimates of predation rates of utility for evaluating predator−prey interactions.

2) Conversion efficiency of energy (21.8%) by lake trout over their 9-yr life in Lake Michigan is about twice that of converting mass to growth (12.2%) due to high-energy fat storage. Failure to consider both ontogenetic increases and seasonal cycles of energy density in both predator and prey can give 2- to 3-fold errors in energy intake estimates based on energetic modeling simulations.

3) Greatest sensitivities of the model to errors of parameter estimation were for parameters relating metabolism to weight, indicating the need for good laboratory studies on metabolism.

4) Highest mass conversion efficiencies are obtained by relatively fast-growing individuals, and a major proportion of energy assimilated annually by older cohorts may be shed as gametes.

5) Peak predatory impact of lake trout occurs 3.5 yr after stocking; peak impact on alewife occurs in the 4th and 5th year, precluding manipulations of stocking densities as a means to control short-term prey fluctuations.

6) Predation by lake trout on alewife has increased steadily since 1965 and further increases are projected for the near future. The model can provide managers with initial indications of possible impacts from these planned increases and various alternative stocking policies.

Acknowledgments

We thank Drs J. F. Kitchell, W. P. Porter, J. E. Breck, J. J.

Magnuson, C. J. Walters, G. Eck, R. Hatch, D. Jester, and an anonymous reviewer for valuable comments and criticism. Laboratory studies on lake trout energetics were conducted at the Great Lakes Fishery Laboratory, U.S. Fish and Wildlife Service, Ann Arbor, Michigan. Modeling studies were part of a Ph.D. thesis in Zoology (Stewart 1980) and a Ph.D. thesis in Water Chemistry (Weininger 1978) at the University of Wisconsin, Madison, Wisconsin. This modeling research was funded by the National Oceanic and Atmospheric Administration, Office of Sea Grant, Department of Commerce, through an institutional grant to the University of Wisconsin. The University of Wisconsin Graduate School provided supplemental computer funds. C. M. Hughes of the Zoology Department, University of Wisconsin-Madison, prepared the illustrations.

A number of colleagues have contributed preliminary data or other information that helped round out this synthesis. They include G. Eck (Great Lakes Fishery Laboratory, U.S.F.W.S., Ann Arbor, MI); D. Jester, R. Rybicki, M. Keller, and L. Moffett (Michigan Department of Natural Resources); R. Poff and J. Pfender (Wisconsin Department of Natural Resources) provided data from studies supported by the Anadromous Fish Conservation Act PL89-304 as part of Wisconsin Project No. AFS-9; S. Yeo (Center for Great Lakes Studies, University of Wisconsin-Milwaukee); and R. Horrall (Laboratory of Limnology, University of Wisconsin-Madison).

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Carotenoids as a Source of Pigmentation in Juvenile Lobsters Fed a Purified Diet[1]

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D'ABRAMO, L. R., N. A. BAUM, C. E. BORDNER, AND D. E. CONKLIN. 1983. Carotenoids as a source of pigmentation in juvenile lobsters fed a purified diet. Can. J. Fish. Aquat. Sci. 40: 699–704.

Pigmentation of cultured lobsters is dependent upon the presence of dietary carotenoids. Inclusion of pure or crude carotenoids in a purified diet results in the accumulation of exoskeleton pigments tentatively identified by thin-layer chromatography and spectrophotometric analysis as primarily astaxanthin and β-carotene. Despite the lack of de novo synthesis pure carotenoids such as β-carotene, echinenone, and canthaxanthin are transformed into astaxanthin. The level of pigmentation produced by these biosynthetic precursors is related to the proximity to the astaxanthin end product. A carotenoid extract from crayfish waste dissolved in cod liver oil, and oleoresin paprika were effective in producing pigmentation. Rationale for the choice and use of carotenoid sources for incorporation into artificial feeds to produce natural pigmentation of cultured lobsters is discussed.

D'ABRAMO, L. R., N. A. BAUM, C. E. BORDNER, AND D. E. CONKLIN. 1983. Carotenoids as a source of pigmentation in juvenile lobsters fed a purified diet. Can. J. Fish. Aquat. Sci. 40: 699–704.

La pigmentation des homards d'élevage dépend de la présence de caroténoïdes dans le régime alimentaire. Si l'on ajoute de ces substances, à l'état pur ou cru, dans un régime purifié, il y a accumulation de pigments exosquelettiques identifiés provisoirement par chromatographie sur couche mince et analyse spectrophotométrique comme étant surtout de l'astaxanthine et du β-carotène. Malgré l'absence de synthèse de novo, des caroténoïdes tels que β-carotène, échinénone et canthaxanthine sont transformés en astaxanthine. Le niveau de pigmentation obtenu par ces précurseurs biosynthétiques est relié à leur proximité du produit final, l'astaxanthine. Un extrait de caroténoïdes de déchets d'écrevisse dissous dans de l'huile de foie de morue et du paprika oléorésineux sont des agents efficaces de pigmentation. Nous examinons les raisons du choix et de l'utilisation de sources de caroténoïdes à incorporer aux régimes artificiels afin de produire, chez les homards d'élevage, une pigmentation naturelle.

Received August 30, 1982
Accepted February 22, 1983

Reçu le 30 août 1982
Accepté le 22 février 1983

CAROTENOIDS are exogenously derived isoprenoid compounds that are responsible for pigmentation in crustaceans. *De novo* synthesis of carotenoids by Crustacea has never been reported. The primary carotenoid found in most crustacean tissue is astaxanthin (Goodwin 1954) which is invariably associated with a protein via ionic bonding. Carotenoid-protein complexes are termed carotenoproteins or chromoproteins and are widely distributed in Crustacea (Cheesman et al. 1967). The function of carotenoids appears multiple. They may act to prevent photooxidation and to stabilize tertiary or quaternary structure in proteins. Carotenoids have also been associated with enhanced growth and reproductive rates, and fecundity in Crustacea and fishes (Tacon 1981; Gilchrist and Lee 1972). In the predatory mite, *Amblyseius potentillae*, dietary carotenoids are required for pigmentation to insure proper response to photoperiodic cues (Van Zon et al. 1981).

Conklin et al. (1983) have emphasized the advantages of an inexpensive ration for the successful commercial culture of lobsters. Provision of a carotenoid source in such a diet will be important in yielding a natural pigmentation acceptable to consumers. The recent development of a purified diet for the successful culture of juvenile lobsters (Conklin et al. 1980) now permits a controlled study of dietary carotenoids as sources of normal pigmentation. In this study we evaluate the effectiveness of pure carotenoids and crude sources of carotenoids for pigmenting cultured American lobsters, *Homarus americanus*.

Materials and Methods

Sibling fourth stage *Homarus americanus* were obtained from the larval rearing facility at the Bodega Marine Laboratory and transferred to individual compartments maintained

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[1] Presented at the 13th Annual Meeting of the World Mariculture Society, Charleston, South Carolina.

Printed in Canada (J7049)
Imprimé au Canada (J7049)

TABLE 1. Composition of purified diet 79F and sources of ingredients.

Ingredient	% Dry weight	Source[b]
Casein	31	(2)
Corn starch	26.6	(1)
Cellulose	1.5	(1)
Soy lecithin — refined	8	(1)
Lipid mix S	6	(3)
Wheat gluten	15	(1)
Vitamin mix BML-2[a]	4	(1)
Spray dried egg white	4	(2)
Mineral mix BTm	3	(4)
Cholesterol	0.5	(1)
Vitamin E acetate 50% (500 IU/g)	0.2	(2)
Vitamin A acetate (500 000 IU/g)	0.1	(2)
Vitamin D_3 (400 000 IU/g)	0.1	(2)

[a] Vitamin mix BML-2 contains: thiamin mononitrate 0.5%, riboflavin 0.8%, nicotinic acid 2.6%, Ca-pantothenate 1.5%, pyridoxine HCl 0.3%, cobalamine 0.1%, folic acid 0.5%, biotin 0.1%, inositol 18%, ascorbic acid 12.5%, PABA 3%, cellulose 60%, BHA 0.1%.

[b] (1) ICN Pharmaceuticals, Inc., Cleveland, OH 44128, USA; (2) Bio-Serv, Inc., P.O. Box 100-B, Frenchtown, NJ 08825, USA; (3) Contains: cod liver oil 66% (Bio-Serv), corn oil 33.8% (Bio-Serv); (4) Mineral Mix Bernhart — Tomarelli modified (ICN).

in a semi-recirculating system described by Conklin et al. (1981). For 80 d, purified diet 79F (Conklin et al. 1980; Table 1) containing no appreciable dietary carotenoids, was fed daily to these animals ad libitum. Small amounts of carotenoids were probably introduced via the cod liver oil and refined soy lecithin ingredients but lobsters fed this diet were essentially devoid of pigmentation. After this pretreatment period, the lobsters were divided into nine separate groups of 13 animals. Seven animals of one group were sacrificed and stored frozen (−20°C) for a baseline analysis; the remainder served as a control for the treatment period. One each of the remaining groups was fed the control diet with either β-carotene, echinenone (this group consisted of only seven animals because of limitations of diet availability), or canthaxanthin, each at 100 mg/100 g of diet, the control diet with oleoresin paprika (100 or 250 mg/100 g of diet; approximately 5 or 12.5 mg total pigment/100 g of diet) or the control diet with crayfish waste extract (1 or 3 mg total pigment/100 g of diet) dissolved in cod liver oil. When a pure carotenoid (0.1%) or the oleoresin paprika (0.1 and 0.25%) was added the amount of dietary lipid mix (Table 1) was reduced to compensate. A final group representing a different progeny were subjected to the same pretreatment period and then fed *Artemia* sp. nauplii (San Francisco Bay Brand), a live laboratory diet that yields excellent growth and pigmentation in juvenile lobsters. The carotenoid composition of *Artemia* sp. is 95−100% canthaxanthin (Krinsky 1965; Soejima et al. 1980).

Diets were prepared by mixing dry ingredients in a Hobart mixer, then adding oils, and blending an additional 15 min. The blended ingredients were combined with approximately 40% (by weight) of cold distilled water and mixed to form a dough-like mass. This dough was cold extruded into 2-mm pellets using a laboratory extruder (Demaco 11 kg/h lab press). All diets were stored frozen (−20°C).

The control and carotenoid containing diets were consumed for 30 d (treatment period). The juvenile lobsters were then starved for 48 h, weighed, and killed for analyses of the carotenoids of the exoskeleton and muscle. Care was exercised to insure that no group sacrificed was disproportionately represented by lobsters in the early part of their molt cycle, a period when pigment lost from the cast exuvium is being accumulated. One intermolt animal from each diet was chosen and placed in boiling water for 2 min to elicit the color change normally associated with cooking. The relative degrees of color change were visually compared. The hepatopancreas and gut were excised from each animal to eliminate any possible pigment contribution from these sources and the total wet weight of each group was then recorded. The carcasses of each group were washed with distilled water and placed in a Virtis homogenizer flask for immediate extraction with 5 vol of cooled acetone. The tissue was homogenized for 5 min under nitrogen in darkness. Homogenates were filtered through a Whatman #1 filter and the resulting filtrate was then refiltered through a 0.5-μm filter. This extraction was repeated and sufficed to remove all discernable color. The two extracts were combined and stored at −20°C under N_2 in darkness for future spectrophotometric analysis.

Calculation of pigment concentration of the acetone extracts of tissue was made according to the known spectral characteristics of astaxanthin in this solvent. Absorbance was measured at 478 nm with a Beckman DU-2 Spectrophotometer and concentration was determined using the extinction coefficient of

$$E_{1\,\mathrm{cm}}^{1\%} = 1600 \quad \text{(Andrewes et al. 1976).}$$

Total quantity of pigment in each extract was determined by multiplying the concentration of a subsample by the total volume of the extract.

Comparison of pigment concentration between the pretreatment group, the *Artemia* fed group and treatment groups could not be based upon total wet weight since lobster weights increase exponentially over time. Weights were changed to surface area using the formula Surface area = $\text{Weight}^{2/3}$, and the tissue concentration of pigment for each group was expressed as $\mu g/cm^2$ of carapace surface area.

The acetone extracted pigments were then transferred to petroleum ether via repeated washings with water. Pure samples of β-carotene and canthaxanthin were dissolved in petroleum ether. Samples of oleoresin paprika and crayfish waste extract in cod liver oil were twice extracted using 5 vol of acetone and transferred to petroleum ether. These pigments were dried over anhydrous sodium sulfate (50 mg/mL) and stored at −20°C under N_2 in darkness. The petroleum ether extracts were concentrated on a rotary evaporator under reduced pressure. The qualitative carotenoid composition of these extracts was then analyzed via thin-layer chromatography (TLC) using Silica gel GHL plates of 250 μm thickness (Analabs, North Haven, CT).

Two solvent systems, (A) petroleum ether/methanol, 95/5 (v/v); and (B) benzene/ethyl acetate/methanol, 75/20/5 (v/v/v); were employed for separation and pure samples of β-carotene and canthaxanthin (Roche Chemical Division, Hoffman La Roche, Inc., Nutley, NJ) were co-

TABLE 2. Average wet weight and pigment content of juvenile lobsters fed purified diets containing pure and crude sources of carotenoids. (n) = number of animals/treatment.

Pure carotenoid or carotenoid source	Dietary pigment concentration (mg/100 g dry weight)	Average wet weight ± SD (mg)	Pigment content (μg/cm^2)	(n)
None (Pretreatment; 80 d)	0.0	269.50±95.02	4.37	(7)
None (Total test period, 110 d)	0.0	474.95±143.85	1.58	(6)
β-carotene[a]	100.0	556.50±99.76	4.29	(12)
Echinenone[b]	100.0	496.86±78.99	12.22	(7)
Canthaxanthin[c]	100.0	567.63±93.00	18.43	(12)
Oleoresin paprika[d]	5.0	581.70±146.70	7.17	(12)
Oleoresin paprika	12.5	593.46±194.75	11.34	(12)
Crayfish waste extract[e] (Astaxanthin)	1.0	535.96±122.43	3.08	(11)
Crayfish waste extract	3.0	557.32±140.38	11.59	(13)
Artemia sp.[f]	4.1−4.4	1098.89±305.10	29.60	(10)

[a] ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, OH 44128, USA.

[b] Obtained from Rex Infanger, Department of Zoology, Brigham Young University, Provo, UT, USA.

[c] Roxanthin Red 10, 10% Dry Canthaxanthin Beadlets, Roche Chemical Division, Hoffman La Roche, Inc., Nutley, NJ 07110, USA.

[d] Meer Corporation, 9500 Railroad Avenue, North Bergen, NJ 07047, USA; H2000 ASTA; added as 100 and 250 mg, pigment concentration approximately 5%, measured as β-carotene.

[e] Obtained from Dr Samuel P. Meyers, Department of Food Science, Louisiana State University, Baton Rouge, LA 70803 USA; pigment concentration = 50 mg/100 g cod liver oil, total pigment measured as β-carotene.

[f] *Artemia* pigment concentration from Soejima et al. (1980); values represent two separate determinations of San Francisco Bay nauplii. Canthaxanthin represented 94.5 and 96.1% of the carotenoids in these samples.

chromatographed. For some compounds complete separation could only be achieved via two dimensional chromatography. R_f values that were different from the available standards were compared with those reported for the same solvent systems and chromatographic material (Castillo and Lenel 1978; Davies 1976).

Carotenoid spots that were in sufficient quantity were then scraped and eluted with petroleum ether (40−65°C b.p.), acetone, or chloroform for additional identification procedures. When possible, the absorption spectra in the visible range (338−650 nm) in one of the aforementioned solvents was determined by using a Beckman DB recording spectrophotometer. Pigment isolation, purification and identification procedures were performed to provide a better understanding of the relationship between dietary and tissue carotenoids. Statistical analysis of the mean wet weights of the treatment groups employed one way ANOVA and multiple comparison Scheffe tests (Zar 1974).

Results

No significant difference in mean wet weights was observed among the treatment groups fed purified diets for the entire 110-d test period. The average wet weight of *Artemia* fed lobsters was significantly greater than that of any other treatment group ($P \le 0.01$).

During the treatment period the pigment content of the lobsters fed the control (carotenoid free) diet decreased (Table 2). These animals were white. In most cases, during the treatment period, the pigment content of the tissue increased in those animals fed diets containing carotenoids. Lobsters fed *Artemia* sp. had the highest pigment level. As a source of canthaxanthin (4.1−4.4 mg/100 g, see Table 2) this live diet is much more effective in producing pigmentation than a purified diet containing the same carotenoid (100 mg/100 g).

The concentration of pigment in the tissue of lobsters fed purified diets containing pure carotenoids increased in the order of β-carotene, echinenone, and canthaxanthin. Only those animals fed diets containing β-carotene or a very low level of crayfish waste extract (1 mg total pigment/100 g diet) had tissue pigment levels which remained the same or decreased slightly from pretreatment levels. Visually, the intensity of natural coloration and the red coloration after boiling paralleled the relative differences in pigment content of the tissue of the lobsters fed the *Artemia* and purified diets.

The diet containing 3 mg/100 g dry weight of carotenoids extracted from crayfish waste and dissolved in cod liver oil produced the best pigmentation relative to dietary concentration. For example 30 times more dietary β-carotene produced only $\frac{1}{3}$ the tissue pigmentation as that of the crayfish extract. The oleoresin paprika additive was also a good source of pigment.

Regardless of the source of dietary carotenoid(s), all lobster tissue had the same qualitative pigment composition (Table 3). Observed R_f values in solvent system A tentatively identified astaxanthin in free and mono-esterified forms to be the primary carotenoid of the lobsters. Other carotenoids present were β-carotene, canthaxantin, and an unidentified compound (R_f = 0.61, max in petroleum ether = 443 and 417 nm). Identification of β-carotene, astaxanthin, astaxanthin ester, and canthaxanthin was supported by absorption spectrum analysis. TLC analysis of the crayfish waste extract in solvent system A, yielded seven carotenoids, astaxanthin and esterified forms of astaxanthin being predominant. The

TABLE 3. R_f values, absorption maxima, and tentative identities of carotenoids in oleoresin paprika, crayfish waste extract, and tissue of juvenile lobsters fed carotenoid containing purified diets or *Artemia*. Solvent systems, A = petroleum ether/methanol, 95/5 (v/v); B = benzene/ethyl acetate/methanol, 75/20/5 (v/v/v). + designates a visual estimate of the relative intensity of a carotenoid compound within the particular sample. (n) = number of different carotenoids observed.

R_f value		Provisional identification	Oleoresin paprika oil (17)	Crayfish extract (7)	Tissue of *Artemia* and diet-fed animals (5)	Absorption maxima (nm)
A	B					
0.98	*	β-carotene	+++	++	+++	470-445-(420)^a
0.96	*	Isocryptoxanthin	++			488-458-(428)^b
0.92	*	β-cryptoxanthin	+++			480-454-(426)^c
0.61	*	?			++	443-417-390^a
0.60		?	+			468-440-(416)^a
0.53		Astaxanthin di-ester		++		466^a
0.46	*	Capsanthin ester	+++			(516)-486^b
0.37		?		+		
0.35		?	++			510-477-(451)^c
0.25		Canthaxanthin		+	+	464^a
0.15	0.64	Capsorubin ester?	+++			500-467-442^a
0.13		Astaxanthin mono-ester		++	++	467^a
0.11	0.59	Lutein	++	+		471-444-420^a
0.07		?	++			
0.04		Free astaxanthin		+++	+++	467^a
0.03	0.54	Zeaxanthin	++			494-462^b
0.02		?	+			
x	0.44	Antheraxanthin	++			472-444-(416)^c
x	0.34	Violaxanthin	+			
x	0.29	Capsanthin	++			
x	0.24	Capsanthin 5,6 epoxide	+			
x	0.21	Capsorubin	+			

[a] In petroleum ether.
[b] In chloroform.
[c] In acetone.
*No resolution.

qualitative composition reflected that previously reported (Wolfe and Cornwell 1965). Oleoresin paprika contained a minimum of 17 carotenoids of which 9 could be tentatively identified from R_f values after using two-dimensional chromatography with solvent systems A and B (Table 3). Provisional identity of β-carotene, β-cryptoxanthin, isocryptoxanthin, zeaxanthin, lutein, and capsanthin was supported by absorption spectrum analysis.

Discussion

A composite scheme for the biosynthesis of carotenoids in crustaceans can be developed from the work of Hsu and Chichester (1970) with *Artemia* sp., Castillo (1980) and Castillo and Lenel (1978) with the hermit crab *Clibanarius erythropus*, Herring (1968) with the cladoceran *Daphnia magna*, and Katayama et al. (1972) with the prawn *Penaeus japonicus* (Fig. 1). Our study demonstrates that lobsters are able to use dietary β-carotene, echinenone and canthaxanthin as precursors in the production of their primary tissue carotenoid, astaxanthin. This capability is consistent with the generalized crustacean biosynthetic scheme of β-carotene → echinenone → canthaxanthin (Fig. 1).

This study indicates that the level of pigmentation achieved at a particular dietary concentration is directly related to the proximity of the carotenoid to the astaxanthin end product in the biosynthetic scheme. These results suggest that the efficacy of a particular crude carotenoid source must be partially related to its qualitative composition.

In our experiment the use of 100 mg/100 g dry weight dietary concentrations of β-carotene, echinenone, and canthaxanthin was arbitrary. Lower concentrations may have been equally effective in the production of the observed pigment level by a pure carotenoid, particularly if the pigment level response to dietary concentration is asymptotic. Exact relationships between dietary concentration of a singular carotenoid source and resulting pigment level remain to be determined.

The pigmentation achieved in lobsters fed *Artemia* suggests that carotenoid provision via a live diet is probably most efficient. However, both the concentration of carotenoids provided by the oleoresin paprika and crayfish waste extract additions to the purified diet and the corresponding levels of pigmentation produced in juvenile lobsters are reasonably comparable to those of the *Artemia* diet.

Success of the crayfish waste extract as a carotenoid source can most probably be attributed to the direct availability of astaxanthin. This is the first report of oleoresin paprika being a good source of pigmentation in Crustacea. Capsanthin and most probably capsorubin are major carotenoid components

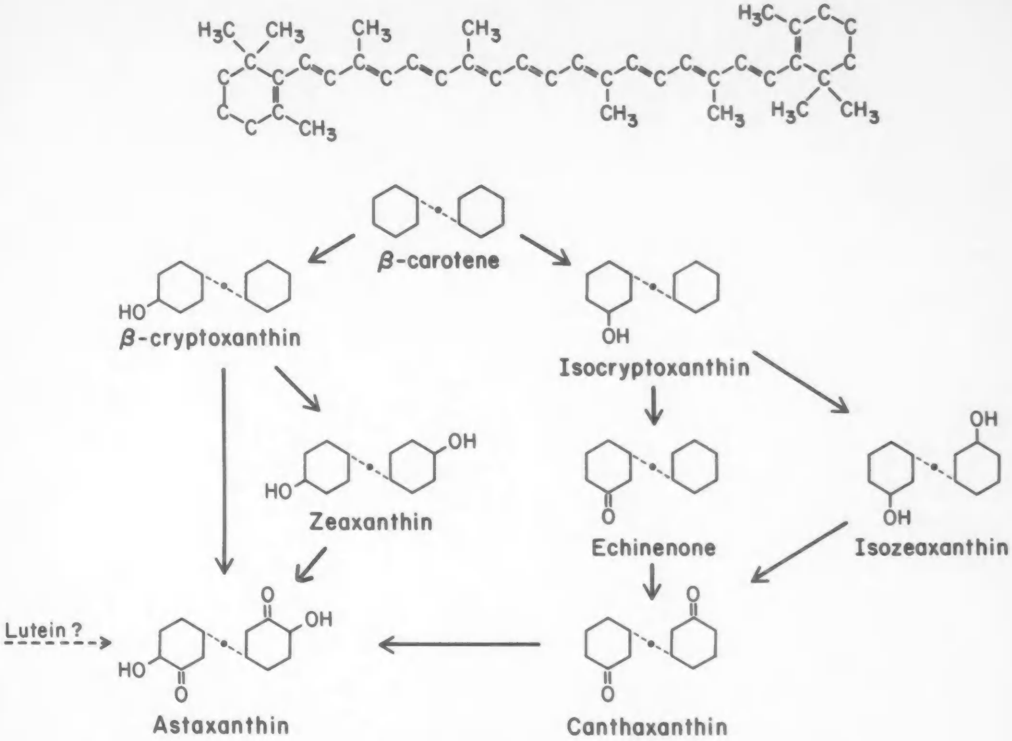

FIG. 1. Biosynthetic pathways used by Crustacea in the transformation of dietary carotenoids.

of this material (Goodwin 1971; see also Table 3). There is no evidence of a reaction where capsanthin or capsorubin is transformed into astaxanthin or a precursor of astaxanthin and these carotenoids have never been reported as pigment in any crustacean tissue. The possibility that these predominant carotenoids of the oleoresin paprika are both physiologically available and transformed by lobsters is presently indeterminate. Also, the effectiveness of the oleoresin paprika may reside in that the other constituent carotenoids, β-carotene, β-cryptoxanthin, isocryptoxanthin, and zeaxanthin, are, as in other crustaceans, intermediaries in the formation of astaxanthin. Dietary isocryptoxanthin can be converted into echinenone and canthaxanthin and isozeaxanthin is readily oxidized to canthaxanthin by the cladoceran brachiopod *Daphnia magna* (Herring 1968). Lutein, another primary component of the oleoresin, may also be involved in the production of astaxanthin in crustaceans as Lenel et al. (1978) have suggested. The uncertainty of which carotenoids can act as precursors to astaxanthin synthesis in lobsters precludes a precise evaluation of the relative effectiveness of the oleoresin paprika based upon total concentration.

Rapidity and intensity of pigment production as well as cost will be an important factor in an ultimate decision regarding what pigment source to include in a commercial feed. Such a source has been elusive. Johnson et al. (1977) had little success with the incorporation of *Phaffia rhodozyma*, a yeast containing astaxanthin as its principal carotenoid (Andrewes et al. 1976), into a purified diet for the lobster. These investigators suggested that the meager consumption of the artificial diet or the unique configuration (stereochemistry) of the astaxanthin molecule derived from *Phaffia* was responsible. Later work with salmonid diets demonstrated that *P. rhodozyma* was an effective source of pigmentation when the cell wall was removed by enzymatic digestion (Johnson et al. 1980).

Efforts to produce coloration in fish fed artificial diets have been quite successful. Schmidt and Baker (1969) fed pink salmon (*Oncorhynchus gorbuscha*), juvenile cutthroat (*Salmo clarki*), and rainbow trout (*S. gairdneri*) diets containing 19−45 mg canthaxanthin/100 g dry weight of diet and successfully produced pigmentation. Dried crustacean waste at 20−30% dry weight of the diet, with and without a canthaxanthin supplement produced pigmentation of brook trout (*Salvelinus fontinalis*) (Saito and Regier 1971). Good pigmentation of brown (*Salmo trutta*), brook, and rainbow trout has previously been achieved with crayfish pigment extracts added at approximately 4.85 mg total xanthophylls/100 g of diet (Peterson et al. 1966). Considerable success was also achieved when the carotenoid source was paprika extract at 23.5 mg total xanthophylls/100 g of diet.

Our work has shown that dried crustacean wastes such as shrimp meal and euphausiid meal incorporated into semipurified diets for lobsters at 18−25% dry weight (Bordner et al. 1980) do not produce adequate pigmentation. Astaxanthin content of the diets using these sources probably ranges from 0.1−0.5 mg/100 g dry weight. Dietary carotenoids derived from crayfish waste extract and composed primarily of astaxanthin appear to be an efficient and inexpensive source of pigmentation in the lobster, particularly when dissolved in a marine derived oil that could be an ingredient of the diet. Dissolution in oil may yield an additional benefit by assisting in maintaining the integrity of the chemical compounds during extended periods of storage. This same pigment source has already been successfully used to produce coloration in salmon (S. P. Myers, Louisiana State University, Baton Rouge, LA, personal communication).

The success of the oleoresin paprika for Crustacea indicates that plant derived carotenoids such as β-cryptoxanthin isocryptoxanthin, and zeaxanthin derived from common sources and dissolved into an oil mix could yield a desirable pigmentation. Depending upon relative effectiveness and concentration such ingredients could be long- or short-term enrichments of an inexpensive ration to assure coloration commensurate with consumer demand.

Acknowledgments

We thank D. C. V. Easterson of the Central Marine Fisheries Research Institute, Cochin, India for his assistance in the TLC and spectrophotometric analysis and Samuel Meyers of Louisiana State University for his continued interest and his gift of the crayfish extract dissolved in cod liver oil. This work is a result of research sponsored in part by NOAA, Office of Sea Grant, Department of Commerce, under Grant No. NA80AA-D-00120.

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Sublethal Effects of Petroleum Hydrocarbons on Adult American Lobsters (*Homarus americanus*)[1]

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A suite of 56 different parameters including biochemical, physiological, and morphological indices were investigated in lobsters chronically exposed to a low level of petroleum hydrocarbons. General condition indices were unaffected and no oil-induced differences were noted in serum values for most of the electrolytes, except calcium, which was elevated in both sexes. Serum freezing-point depression decreased in both sexes of the experimental group. Considering the four major serum parameters, protein, lipid, glucose, and amino nitrogen, oil exposure had a significant effect on plasma protein and lipid values in the females. Chronic exposure to oil also had a marked impact on blood hemocytes in which lipid values were reduced by approximately one-half in both sexes. Three different types of oil-induced effects were noted for the essential amino acids, including (a) change in one sex only, (b) change in both sexes, and (c) interaction between sex and oil exposure. The only nonessential amino acid significantly affected by oil exposure was alanine. The differences observed in amino acid analogs, namely the elevation of serum levels of methionine sulfoxide and methylhistidine, have been discussed on the basis of mammalian studies as possible stress-related effects. One of the most interesting effects observed at the organismal level was gill browning, which markedly increased in the oil-exposed animals. This particular sublethal effect, possibly more than any other, could be considered pathological in nature. Gill browning is readily measurable and provides one suitable biological response for monitoring point sources of petroleum pollution in lobster habitats.

PAYNE, J. F., J. KICENIUK, R. MISRA, G. L. FLETCHER, AND R. J. THOMPSON. 1983. Sublethal effects of petroleum hydrocarbons on adult American lobsters (*Homarus americanus*). Can. J. Fish. Aquat. Sci. 40: 705−717.

On a étudié, sur des homards exposés de façon chronique à de faibles niveaux d'hydrocarbures de pétrole, une série de 56 paramètres différents, y compris indices biochimiques, physiologiques et morphologiques. Les indices de condition générale ne sont pas influencés et aucunes différences dues au pétrole n'ont été notées dans les valeurs de la plupart des électrolytes du sérum, sauf le calcium, qui est élevé dans les deux sexes. L'abaissement du point de congélation du sérum est moindre dans les deux sexes du groupe expérimental. Si l'on considère les quatre principaux paramètres du sérum, protéines, lipides, glucose et aminoazote, l'exposition au pétrole a un effet marqué sur les protéines et les lipides du plasma des femelles. Une exposition chronique au pétrole a également une influence significative sur les hémocytes sanguins dans lesquels les valeurs diminuent d'environ la moitié dans les deux sexes. Dans le cas des acides aminés essentiels, on note trois types différents d'effets provoqués par le pétrole, y compris (a) changement dans un sexe seulement, (b) changement dans les deux sexes et (c) interaction entre sexe et exposition au pétrole. L'alanine est le seul acide aminé non essentiel qui soit affecté de façon significative par le pétrole. Les différences observées dans les analogues d'acides aminés, soit l'élévation des niveaux sériques de sulfoxyde de méthionine et l'histidine-méthyl, ont été analysées à la lumière d'études sur les mammifères, comme étant possiblement des effets liés au stress. Un des effets les plus intéressants qui ait été observé au niveau organismique est le brunissement des branchies, qui

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[1] Marine Sciences Research Laboratory Contribution No. 441.

Printed in Canada (J6893)
Imprimé au Canada (J6893)

augmente considérablement chez les sujets exposés au pétrole. Cet effet sublétal particulier, peut-être plus que tout autre, serait considéré comme étant de nature pathologique. Ce brunissement est facilement mesurable et constitue une réaction biologique permettant de surveiller des sources ponctuelles de pollution par le pétrole dans les habitats du homard.

Received April 7, 1982
Accepted February 25, 1983

Reçu le 7 avril 1982
Accepté le 25 février 1983

BECAUSE of problems incurred in establishing the effects of pollutants on aquatic organisms at population and community levels of biological organization (e.g. Cushing 1979; Spies et al. 1980), it is becoming generally accepted that "biological indicator" studies may be of value in assessing the possible impact of various environmental stressors on aquatic animal health (Lockhart et al. 1972; Donaldson and Dye 1975; Wedemeyer and Yasutaki 1977; McLeay 1977; Thomas et al. 1980; Gould 1980). It is also hoped that indices suitable for biologically monitoring animal populations might eventually be developed (MacIntyre and Pearse 1980), and a few have been assessed in the field (e.g. Payne 1976; Stegeman 1980; Hodson et al. 1980; Lee 1981a). It is recognized, however, that the most that can be accomplished by the use of such indices is to suggest that a portion of the individuals of any one species is probably not healthy.

The short-term effects of petroleum hydrocarbons on aquatic crustaceans have been well documented (e.g. Anderson 1975; Lee et al. 1978; Laughlin et al. 1978; Johns and Pechenik 1980) and both Wells and Sprague (1976) and Percy (1978) have observed acute as well as chronic sublethal effects in larval crustaceans chronically exposed to petroleum.

Unlike fish, crustaceans such as lobster may have a limited capacity to metabolize the polycyclic organic compounds found in large concentrations in petroleum (Lee et al. 1977; Payne and May 1979; Lee 1981b), and Vandermeulen et al. (1980) recently observed that toxic petroleum derivatives such as naphthalene are rapidly bioconcentrated in the hemolymph of adult *Cancer* crabs.

We have investigated a variety of morphological, physiological, and biochemical responses in adult lobsters chronically exposed to a low level of petroleum hydrocarbons. Such a suite of indices, which might provide useful indicators of toxic stress and/or suitable monitoring parameters, has not been studied in any crustacean species to date. Lobsters were also selected for this study because they are one of the most valuable commercial species found along the northeast coast of the United States and in Atlantic Canada (Gordon de Wolf 1974), and many populations are found in inshore waters, the type of environment likely to be most susceptible to any major oil discharge.

Methods and Materials

Newly trapped American lobsters (*Homarus americanus*) from Placentia Bay, Newfoundland, were held in flow-through seawater aquaria at the Marine Sciences Research Laboratory, Memorial University. Individual animals were weighed and divided into two equal groups, each of which was held in 240-L aquaria (1 × 1 × 0.3 m) continuously supplied with seawater (approximately 2 L/min) maintained at the ambient temperature (4–13°C) and photoperiod experienced from May to August. Ceramic drain tiles were used to provide individual shelters for the animals. Venezuelan crude oil (100 mL) was added to one aquarium and the oil slick was restricted to approximately one-third of the water surface. Animals were fed herring (*Clupea harengus*) or capelin (*Mallotus villosus*) ad libitum. The lobsters were dosed weekly for 14 wk, the "weathered" oil being removed at each period (Payne et al. 1978). Oil was first skimmed from the surface and the standpipe was then removed for approximately 5 min to permit discharge of the surface oil sheen.

The acute toxicity of petroleum is believed to be due to the relatively soluble monoaromatic and diaromatic type compounds (Anderson et al. 1974; Rossi et al. 1976), and the experimental plan was to closely simulate an environmental situation in which animals would be exposed to relatively low levels of "water-soluble" hydrocarbons. The oil used in the experiment was a medium light Venezuelan crude (commonly transported in Atlantic Canada), and class analysis by high-pressure liquid chromatography (Beckman model 110 using an energy analysis column from Waters Associates) indicated that it contained approximately 200 mg/g monoaromatics, 12 mg/g diaromatics, and 3 mg/g triaromatics.

Total oil concentrations in the experimental tank measured by fluorescence spectroscopy (Keizer and Gordon 1973) ranged from approximately 300–500 μg/L on day 1 to 40–60 μg/L on day 6 (four trials). This is a relatively low concentration of petroleum hydrocarbons, which includes the water-soluble component plus any dispersed microparticulates generated by airstone turbulence, animal movement, and possibly emulsification of oil by fatty food particles. The actual "water-soluble" concentrations in the experiment would be expected to be considerably lower. Gentle mixing of oil over seawater in beakers has been shown to generate water-soluble concentrations in the 1–10 mg/L range (Rice et al. 1977), which can be assumed to approximate seawater saturation levels. A similar gentle mixing of oil in our flow-through system (in the absence of animals and without evidence of airstone-mediated emulsification) generated very low levels of total soluble hydrocarbons as measured by fluorescence spectroscopy: approximately 20 μg/L after 2 h (six different trials in duplicate), 30 μg/L after 24 h (two different trials in duplicate), and 8 μg/L after 48 h (two different trials in duplicate). Beyond this time, i.e. at 72, 120, and 160 h, the levels obtained (1–4 μg/L) were generally indistinguishable from background levels.

Waste product accumulation (e.g. ammonia) should not have been a problem in the experiment, since the water turnover rate was quite high, approximately 2 L/min. Both aquaria were also vigorously aerated to obviate chance of oxygen debt. Salinity measurements were not taken throughout the course of the experiment, but Kendaris (1980) observed that even during spring and summer runoff months, the salinity of nearshore waters in Logy Bay (the site of the Marine Sciences Research Laboratory) remains quite constant, approximately 32‰. In this regard, it is also worth noting that Logy Bay is situated on an exposed headland just north of St. John's and receives water from the Labrador Current.

At the end of the exposure period, blood samples were taken and the lobsters were weighed and necropsied. Hemolymph was removed from the abdominal sinus using plastic syringes fitted with 21-gauge needles. The first 0.5 mL of hemolymph was drawn into a 3-cm^3 syringe containing 40 mL of a seawater solution (0.5%) containing 40 mmol/L *N*-ethylmaleimide, which was used to prevent hemocyte aggregation. This blood sample, further diluted in filtered seawater containing 40 mmol/L *N*-ethylmaleimide, was used to count and size the cells using a Coulter counter (model ZB) equipped with a channelyzer. The remaining hemolymph was centrifuged to remove the blood cells. The plasma was divided into three portions, one for amino acid analysis, one for electrolyte analysis, and one for plasma lipid, glucose, protein, and amino nitrogen determination. The samples used for amino acid and electrolyte analyses were heated at 60°C for 60 s to prevent clotting of the plasma.

Concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, and Zn^{2+} in the plasma were determined by atomic absorption (Varian Tectron model AA5), and Cl^- was determined by titration (Radiometer model CMT 10) (Fletcher and King 1978). Plasma freezing-point depression was determined using an Advanced Osmometer (model 3D), and inorganic phosphorus was determined by the method of Fiske and Subbarow (1925) (kit No. 670-C, Sigma Chemical Co., St. Louis, MO). Plasma and muscle water were determined by drying weighed aliquots at 90°C for 7 d.

Plasma glucose was determined by the glucose oxidase method, and plasma amino nitrogen was determined by the fluorescamine procedure (Udenfriend et al. 1972). Plasma lipid, plasma protein, and hemocyte constituents were estimated as described by Thompson (1977). Free amino acids were determined on deproteinized plasma using a Beckman model 121 amino acid analyzer (Squires et al. 1976). The physical characteristics of all the organs were carefully examined. Both gill browning and ovary greenness were estimated as a percentage of the total organ surface.

STATISTICAL ANALYSIS

Data were grouped as follows: control males (CM), control females (CF), males exposed to oil (EM), and females exposed to oil (EF). Up to 56 physiological and morphological characters were measured on each individual. The differences between sexes (M−F), between treatment conditions (C−E), and their interactions (M−F) × (C−E) were analyzed. Analysis of variance (ANOVA) was employed on a cross-classification (sex × treatment) layout. Nonorthogonality of data caused by unequal and disproportionate numbers of individuals in the design was duly accounted for in ANOVA. At first, each variable of a group was considered separately by univariate ANOVA. This was followed by multivariate analysis. Wilks lambda values (e.g. Kshirsagar

TABLE 1. Effect of petroleum on various condition indices in lobsters (a, aa, aaa, significantly different at $P < 0.05$, 0.025, and 0.01, respectively; B, BB, BBB, significantly different in the Bonferroni procedure at $P < 0.05$, 0.025, and 0.01, respectively; M, male; F, female; C, control; E, exposed to oil).

		Control			Exposed			Statistical analysis						
Variable	Sex	Mean	SD	*N*	Mean	SD	*N*	(M−F)C	(M−F)E	(M−F)	(C−E)M	(C−E)F	(C−E)	(M−F) × (C−E)
Body weight (g)	M	511	71.4	13	561	71.8	14							
	F	499	45.5	17	515	61.5	14							
Ovary weight (g)	M													
	F	105	26.8	17	100	29.3	13							
Liver weight (g)	M	28.9	4.26	13	30.4	5.02	14	aM/F	aaaM/F	aaaM/F				
	F	25.5	3.53	17	26.1	3.93	14							
Gill browning (arcsine √%)	M	15.2	6.01	13	27.2	9.40	14				aaaE/C	aaaE/C	aaaE/C	
	F	15.6	5.13	17	28.2	13.3	14				BBB	BBB		
Legs	M	7.8	0.60	13	7.8	0.58	14							
	F	7.8	0.73	17	7.4	1.45	14							
Ovary greenness (arcsine √%)	M													
	F	35.9	15.7	17	39.1	18.2	13							

TABLE 2. Effect of petroleum on lobster plasma constituents (a, aa, aaa, significantly different at $P < 0.05, 0.025$, and 0.01, respectively; B, BB, BBB, significantly different in the Bonferroni procedure at $P < 0.05, 0.025$, and 0.01, respectively; M, male; F, female; C, control; E, exposed to oil).

		Control			Exposed			Statistical analysis						
Variable	Sex	Mean	SD	N	Mean	SD	N	(M−F)C	(M−F)E	(M−F)	(C−E)M	(C−E)F	(C−E)	(M−F) × (C−E)
Plasma protein (mg/mL)	M	59.9	14.0	13	58.1	10.1	14	aaaF/M	aaaF/M	aaaF/M		aC/E		
	F	88.8	9.25	17	78.7	18.6	14	BBB	B					
Plasma glucose (mg/100 mL)	M	1.27	0.691	13	1.41	0.442	14							
	F	1.58	0.647	17	1.67	0.480	14							
Plasma amino nitrogen (mg/100 mL)	M	37.5	9.53	9	34.3	6.69	11	aaaE/M	aaaF/M	aaaF/M				
	F	54.5	18.8	14	50.5	9.06	12							
Plasma lipids (mg/100 mL)	M	146	33.3	9	127	34.2	11	aaaF/M	aaaF/M	aaaF/M		aC/E		
	F	1290	484	14	998	415	12	BBB	BBB					

1972) were estimated for comparisons between M−F, C−E, and their interactions.

Results and Discussion

Lobster in the control and oil-contaminated aquaria were observed feeding throughout the experimental period and there was no obvious visual difference in food consumption between the two groups. It was estimated that more than 90% of the excess food was removable at each feeding but a quantitative feeding budget could not be carried out, since this would have involved tank drainage and removal of both lobsters and shelters at each feeding period. It is of particular interest that oil exposure did not cause any reduction in body, hepatopancreas, and ovary weights (Table 1). Lobsters lose muscle and hepatopancreas lose weight when starved for relatively brief periods of time (Stewart et al. 1967); therefore, the results of the experiment (viz. observations on body, hepatopancreas, and ovary weights) also indicate that food consumption between the control and oiled groups was comparable. This is in contrast with observations on fish, in which food intake was considerably reduced upon exposure to crude oil resulting in decreases in growth and gonad weight (Fletcher et al. 1981).

Although no carcasses were observed, 10 lobsters were lost from the control tank and 11 from the experimental tank. Lobsters are known to be cannibalistic and the remnants of hard shell occasionally retrieved from the aquaria indicated that dead lobsters were consumed. Animal loss may not have been due to "natural" mortality but due to cannibalism during moult. The experiment covered part of the molting cycle, from late July to early August, and lobsters in other tanks at the Marine Sciences Research Laboratory were observed molting during this period.

The bright green color of the ovaries and plasma of female lobsters suggest that vitellogenesis was taking place (Schade and Shivers 1980). Female lobsters had significantly higher concentrations of plasma proteins, lipids, phosphorous, zinc, and amino nitrogen and a lower percentage of water than did male lobsters (Tables 2−4). It is likely that the higher protein and lipid values and lower percentage of water in the females were due to the presence of vitellogenin. The high phosphorus concentration in females may also reflect the vitellogenin level, since the acid conditions used to precipitate plasma proteins could have caused the hydrolysis of phosphate esters (Henry 1964). Recently, zinc has been found to be associated with vitellogenin in fish (Fletcher and Fletcher 1980); therefore, it is also possible that the high zinc levels in female lobster are due to the presence of this particular lipoprotein.

ELECTROLYTES AND MAJOR SERUM PARAMETERS

Male lobsters had significantly higher concentrations of plasma Na^+ and Cl^- than did females (Table 3). This difference would amount to a freezing-point depression of 0.04−0.06°C if all the Na^+ and Cl^- were osmotically active. Since plasma freezing-point depression did not differ between sexes (Table 4), it may be that during vitellogenesis the females contain increased concentrations of osmotically active compounds, such as amino nitrogen, which would

TABLE 3. Effect of petroleum on lobster electrolytes (a, aa, aaa, significantly different at $P < 0.05$, 0.025, and 0.01, respectively; B, BB, BBB, significantly different in the Bonferroni procedure at $P < 0.05$, 0.025, and 0.01, respectively; M, male; F, female; C, control; E, exposed to oil).

		Control			Exposed			Statistical analysis						
Variable	Sex	Mean	SD	*N*	Mean	SD	*N*	(M−F)C	(M−F)E	(M−F)	(C−E)M	(C−E)F	(C−E)	(M−F) × (C−E)
Calcium (μg/mL)	M	604	41.3	13	652	86.2	14					aE/C		
	F	604	50.3	17	631	68.5	14							
Magnesium (μg/mL)	M	165	15.6	13	166	17.7	14							
	F	158	10.4	17	166	17.5	14							
Sodium (mmol/L)	M	464	6.69	13	465	4.62	14	aaaM/F	aaaM/F	aaaM/F				
	F	445	6.11	17	447	11.4	14	BBB	BBB					
Potassium (mmol/L)	M	5.1	0.81	13	5.0	0.50	14							
	F	4.8	0.56	17	5.1	0.62	14							
Copper (μg/mL)	M	62.1	20.5	13	63.3	15.2	14							
	F	61.9	13.0	17	60.6	13.9	14							
Zinc (μg/mL)	M	9.5	1.50	13	10.5	1.92	14	aaaF/M	aaaF/M	aaaF/M				
	F	14.7	2.22	17	14.7	2.43	14	BBB	BBB					
Phosphorus (μg/mL)	M	9.6	2.36	13	8.6	2.05	14		aaaF/M	aaaF/M				
	F	10.9	2.60	17	11.7	2.13	14		B					
Chloride (mmol/L)	M	474	16.2	13	477	11.2	14			aM/F				
	F	457	19.2	17	466	11.9	15							

TABLE 4. Effect of petroleum on plasma water, muscle water, and freezing-point depression (a, aa, aaa, significantly different at $P < 0.05$, 0.025, and 0.01, respectively; B, BB, BBB, significantly different in the Bonferroni procedure at $P < 0.05$, 0.025, and 0.01, respectively; M, male; F, female; C, control; E, exposed to oil).

		Control			Exposed			Statistical analysis						
Variable	Sex	Mean	SD	*N*	Mean	SD	*N*	(M−F)C	(M−F)E	(M−F)	(C−E)M	(C−E)F	(C−E)	(M−F) × (C−E)
Freezing point (°C)	M	1.69	0.035	13	1.66	0.028	13				aaaC/E	aaC/E	aaaC/E	
	F	1.68	0.024	17	1.66	0.019	14							
Plasma water (%)	M	92.0	1.12	13	92.2	0.98	14	aaaM/F	aaaM/F	aaaM/F				
	F	88.8	1.34	17	89.7	1.51	14	BBB	BBB					
Muscle water (%)	M	78.9	1.13	13	78.7	0.93	14							
	F	78.9	0.97	17	79.3	1.15	14							

account for the 0.04−0.06°C difference.

No oil-induced differences were noted in serum values for most of the electrolytes, including sodium, potassium, chloride, zinc, copper, and phosphorus. Some of these electrolytes have been shown to be affected by various types of stress in a number of other species. One of the most commonly studied electrolytes, chloride, has been observed to decrease in both fish (Thurberg et al. 1978; Payne et al. 1978) and bivalves (Anderson 1977) exposed to oil, as well as in shrimp exposed to polychlorinated biphenyls (Anderson 1977) and crabs exposed to elevated water temperatures (Lynch et al. 1973). The common observation of serum chloride depression in various species and under different types of chemical and physical stress was not supported in this study with lobster.

Calcium was the only electrolyte shown to be significantly affected by chronic oil exposure, and it was elevated in both sexes (Table 3). The importance of calcium in shell formation in crustaceans is well recognized. Since serum Ca^{2+} concentrations increase in lobster just prior to molting (Travis 1955), any stress-mediated elevation of this electrolyte may lead to (or represent) asynchrony of the normal molting cycle. The direct role of calcium in modulating lobster heartbeat should also be noted (Prosser 1969).

It is of particular interest that of the four major serum parameters (protein, amino nitrogen, lipid, and glucose), oil exposure had the greatest effect on plasma protein and lipid values in the females (Table 2). Although not statistically significant, the mean value for plasma water was higher in the oiled group in proportion to the protein concentration decrease (Table 4). Male lobsters showed no significant differences in this regard, nor was there any indication of even a slight disturbance in plasma protein values. This may suggest that oil exposure interfered with vitellogenesis either by decreasing the rate of vitellogenin production or by increasing its rate of clearance from the plasma.

Oil exposure caused a significant reduction in plasma freezing-point depression (Table 4). The results obtained here with lobsters ("osmoconformers") compare favorably with results for fish ("osmoregulators") collected near the Argo-Merchant spill site (Thurberg et al. 1978). Reduction in freezing-point depression (0.04−0.06°C) could be the result of a comparable decline in osmotically active plasma constituents or an increase in plasma water. However, the concentrations of major plasma electrolytes and plasma water did not appear to differ between treatments (Tables 3 and 4). It is possible that oil exposure increased the degree to which dissolved substances were associated with one another, reducing the freezing-point depression accordingly.

HEMOCYTES

Male lobsters had higher numbers of hemocytes (Table 5), but hemocytes from female lobsters contained elevated concentrations of lipid, carbohydrate, and protein. Lipid bodies have been observed in one type of hemocyte in blue crabs (Bodammer 1978), and in molluscs, Thompson (1977) suggested that hemocyte lipid may play a role in oogenesis. Thus, the differences in hemocyte composition between male and female lobster may be a reflection of their reproductive physiology.

Although chronic oiling had no effect on cell numbers, distinct hemocyte effects were noted. A marked decrease in cell lipid in the absence of any change in cell carbohydrate or protein was observed in both sexes. A reduction in cell lipid of approximately 50% suggests that crude oil exposure effected a considerable change in lipid metabolism. Any major change in hemocyte physiology may be of special importance, since these cells are believed to participate in such critical functions as disease defense (Robin 1970).

ESSENTIAL AND NONESSENTIAL AMINO ACIDS

Sex differences were noted for a relatively few essential and nonessential amino acids (Table 6 and 7). Plasma concentrations of the essential amino acid, tryptophan, and the nonessential amino acid, serine, were significantly greater in control males than in control females (Tables 6 and 7). The nonessential amino acids, alanine and cystine, were significantly higher in males than in females in both control and oil-exposed groups (Table 7). These sex differences may reflect the female lobster's greater requirements for amino acids during vitellogenesis.

There were a number of indications that oil exposure resulted in disturbances in amino acid metabolism. Three types of effects were noted: (a) difference in one sex only, (b) difference in both sexes, and (c) interaction between sex and oil exposure. Of the three aromatic amino acids, tryptophan, phenylalanine, and tyrosine, tryptophan levels decreased in males exposed to oil (Table 6). Tryptophan deficiency in mammals can directly alter levels of niacin, the critical vitamin for biological oxidation processes (Meister 1965a). But no change was noted for any of the branched chain amino acids, leucine, valine, and isoleucine (Table 6), which are associated with increased protein catabolism during injury stress in mammals (Wedge et al. 1976).

Methionine increased in both sexes of the oil-exposed group, but the remaining sulfur amino acid, cystine, did not display a significant difference (Table 7). Many varied biological functions have been described for methionine, including a role as methyl group donor in phenol detoxification (Brown 1976). Petroleum is rich in phenols, and the increased methionine in the oil-exposed lobster may reflect an involvement in the metabolism of such compounds.

Nonessential amino acids have been reported to be of prime importance in cell osmoregulation (Gerard and Giles 1972). Alanine was the only nonessential amino acid to be affected by oil exposure, and it was elevated in both sexes (Table 7). This is of interest, since this particular amino acid is believed to play a key role at the metabolic crossroads of glycolysis and gluconeogenesis (Scrutton and Utter 1968). Although an understanding of the regulation of these processes is incomplete, current evidence indicates that increased alanine levels can assure the inhibition of a critical enzyme (Weber 1969) directing amino acid flow to glucose. This process may become operative under conditions of carbohydrate restriction or any stress-mediated cell catabolism in which amino acids are being utilized for energy. The increase in alanine levels in oiled lobsters may indicate the operation of such a process albeit at a level insufficient for actual loss of body and organ weights.

TABLE 5. Effect of petroleum on lobster hemocytes (a, aa, aaa, significantly different at $P < 0.05$, 0.025, and 0.01, respectively; B, BB, BBB, significantly different in the Bonferroni procedure at $P < 0.05$, 0.025, and 0.01, respectively; M, male; F, female; C, control; E, exposed to oil).

		Control			Exposed			Statistical analysis						
Variable	Sex	Mean	SD	N	Mean	SD	N	(M−F)C	(M−F)E	(M−F)	(C−E)M	(C−E)F	(C−E)	(M−F) × (C−E)
Blood cell volume (μm^3)	M	611	273	13	521	286	14							
	F	522	255	17	612	182	14							
Cell carbohydrate (mg/g wet wt)	M	2.32	1.16	9	2.11	0.93	11	aF/M	aaF/M	aaaF/M				
	F	3.81	1.93	14	3.97	2.17	12		B					
Cell lipid (mg/g wet wt)	M	6.63	7.13	10	2.74	1.61	11	aaF/M		aaaF/M	aC/E	aaaC/E	aaaC/E	
	F	11.3	4.71	14	6.0	2.35	12	BBB				BBB		
Cell protein (mg/g wet wt)	M	76.4	29.9	9	70.0	16.4	11	aaF/M	aF/M	aaaF/M				
	F	105	31.4	14	93.1	21.7	12							
Cell number/mm^3	M	19 300	8670	10	16 400	6440	11			aM/F				
	F	13 500	4450	14	14 700	3880	12							

TABLE 6. Effect of petroleum on essential amino acids of lobsters (a, aa, aaa, significantly different at $P < 0.05$, 0.025, and 0.01, respectively; M, male; F, female; C, control; E, exposed to oil).

		Control			Exposed			Statistical analysis						
Variable (nmol/mL)	Sex	Mean	SD	*N*	Mean	SD	*N*	(M−F)C	(M−F)E	(M−F)	(C−E)M	(C−E)F	(C−E)	(M−F) × (C−E)
Isoleucine	M	27.2	15.2	13	29.3	10.7	11							
	F	34.0	22.3	16	43.5	36.3	14							
Phenylalanine	M	9.4	4.98	13	6.3	2.96	11							
	F	9.4	3.56	26	9.8	8.28	14							
Lysine	M	33.1	18.8	13	30.0	10.5	11							
	F	38.3	24.0	16	34.5	25.0	14							
Leucine	M	38.6	21.6	13	40.2	14.6	11							
	F	46.0	25.7	16	55.8	48.7	14							
Threonine	M	33.0	25.4	13	30.1	13.6	11							
	F	32.6	16.0	16	31.5	17.3	14							
Valine	M	36.6	27.7	13	43.2	19.3	11							
	F	40.8	21.9	16	54.1	41.6	14							
Histidine	M	14.1	6.71	13	10.0	5.94	11							
	F	10.9	4.54	16	13.6	5.97	14							a
Tryptophan	M	5.9	7.23	13	2.0	2.80	11	aaM/F			aC/F			
	F	10.9	3.35	16	2.2	3.22	14							
Arginine	M	32.1	13.7	13	23.8	5.88	11							a
	F	27.0	9.06	16	31.3	11.0	14							
Methionine	M	54.5	21.6	13	69.9	19.4	11						aE/C	
	F	48.6	23.1	16	63.7	33.9	14							

TABLE 7. Effect of petroleum on nonessential amino acids of lobsters (a, aa, aaa, significantly different at $P < 0.05$, 0.025, and 0.01, respectively; M, male; F, female; C, control; E, exposed to oil).

								Statistical analysis						
		Control			Exposed									(M−F) × (C−E)
Variable (nmol/mL)	Sex	Mean	SD	*N*	Mean	SD	*N*	(M−F)C	(M−F)E	(M−F)	(C−E)M	(C−E)F	(C−E)	
Aspartic acid	M	3.6	1.05	13	3.9	1.16	11							
	F	3.6	1.72	16	3.6	1.61	14							
Serine	M	55.4	24.8	13	48.1	17.2	11	aaM/F						
	F	38.7	13.5	16	44.9	16.6	14							
Glutamine	M	235	65.3	13	268	67.9	11							
	F	229	59.0	16	236	84.7	14							
Asparagine	M	17.7	10.6	13	20.5	5.55	11							
	F	16.0	8.22	16	16.9	11.9	14							
Tyrosine	M	8.5	6.8	8	13.9	4.49	4							
	F	12.3	16.9	9	17.4	15.2	7							
Alanine	M	189	44.3	13	218	41.0	11	aM/F	aM/F	aaaM/F			aE/C	
	F	152	49.5	16	176	43.4	14							
Cystine	M	20.9	7.00	13	20.7	4.35	5	aM/F	aaM/F	aaaM/F				
	F	13.0	10.98	9	8.5	6.26	7							
Glutamic acid	M	1.3	1.83	0.3	1.08	11.0								
	F	1.2	1.54	16	1.6	2.87	14							
Glycine	M	81.7	45.3	13	76.1	21.8	11							
	F	75.5	23.7	16	90.2	22.4	14							
Proline	M	616	216	13	616	177	11							
	F	599	126	16	660	229	14							

TABLE 8. Effect of petroleum on amino acids analogs (a, aa, aaa, significantly different at $P < 0.05$, 0.025, and 0.01, respectively; B, BB, BBB, significantly different in the Bonferroni procedure at $P < 0.05$, 0.025, and 0.01, respectively; M, male; F, female; C, control; E, exposed to oil).

		Control			Exposed			Statistical analysis						
Variable (nmol/L)	Sex	Mean	SD	*N*	Mean	SD	*N*	(M−F)C	(M−F)E	(M−F)	(C−E)M	(C−E)F	(C−E)	(M−F) × (C−E)
Taurine	M	27.8	13.6	13	26.3	10.0	11							
	F	26.5	7.97	16	27.1	15.7	14							
α-Aminoadipic acid	M	2.8	3.72	13	2.0	3.66	11							
	F	0.7	1.81	16	2.1	3.63	14							
α-Amino-*n*-butyric acid	M	8.7	6.30	13	6.1	2.30	11							
	F	8.7	4.02	16	8.8	4.17	14							
Citruline	M	3.0	4.25	13	2.9	2.57	11							
	F	1.4	2.36	16	3.6	3.12	14							
Cystathionine	M	22.7	11.0	13	19.7	3.53	7		aaaF/M	aaaF/M				
	F	29.5	11.2	16	36.9	12.7	9		B					
Ornithine	M	2.3	2.79	13	3.5	2.59	11							
	F	2.1	1.96	16	2.9	2.76	14							
Ammonia	M	114.9	31.2	13	119	37.7	11							
	F	113.9	24.8	16	129	27.9	14							
1-Methylhistidine	M	1.5	1.55	13	2.2	1.74	11			aM/F				
	F	0.8	1.29	16	0.9	2.10	14							
3-Methylhistidine	M	2.2	2.24	13	3.1	2.57	11			aM/F		aE/C	aE/C	
	F	1.5	1.19	16	3.6	3.50	14							
Methionine sulfoxide	M	0.0	0.00	13	5.3	11.6	11				aE/C		aaE/C	
	F	0.3	1.22	16	3.2	5.41	14							

Some amino acid analogs showed significant sex differences, while others were affected by oil exposure. Cystathionine was higher in females while 1-methylhistidine and 3-methylhistidine were higher in males (Table 8). Both methionine sulfoxide and 3-methylhistidine were significantly higher in oil-exposed lobsters (Table 8). The presence of enhanced levels of methylhistidine may indicate a form of catabolic stress, since this analog appears to be a good indicator of the breakdown of myofibrillar protein in mammals (Nishizawa et al. 1979). The presence of enhanced levels of methionine sulfoxide is also of interest. Protein methionine groups can undergo nonenzymatic oxidation to methionine sulfoxide, but an enzymatic reduction system may counter this debilitative process (Meister 1965b). Enhancement of methionine sulfoxide levels may reflect "aged" or stressed tissues in which critical protective mechanisms are impaired.

GILL BROWNING

One of the most striking differences observed was the increased gill pigmentation (gill browning) in the oil-exposed group (Table 1). Such gill pigmentation has been observed in shrimp taken from the Gulf of Mexico (Couch 1978), and laboratory studies have indicated that other stressors, e.g. cadmium, can produce "black gill syndrome" (Nimmo et al. 1977). This condition has also been associated with protozoan and fungal infections in shrimp (Couch 1978), suggesting a general cell stress etiology. Couch (1978) suggested that gill browning may result from death and necrosis of epithelial tissue (eventually followed by fungal infection) resulting in a dysfunction in respiration and osmoregulation. Histological studies of lobster tissue (unpublished observations) indicated various stages of gill filament occlusion, and it seems reasonable to suggest that hemolymph channel blocking could impair gill function. It is possible that gill pigmentation reflects a process of normal cell debilitation or "ageing," which is enhanced under conditions of cell stress, such as oil or cadmium exposure.

Gill browning may be a common response to gill tissue damage and has appeal as a biological indicator for use in crustaceans populations. Unlike many biochemical–physiological effects, it is the type of response that would be generally acceptable as pathological in nature and thus of special ecological significance. Also, it is easily measurable. More specifically, gill browning may offer a suitable biological response for monitoring point sources of marine pollutants such as petroleum hydrocarbons. Candidate sources of hydrocarbons could be heavily contaminated coastal sediments, offshore production zones, or refinery outfall areas. Monitoring would entail carrying out periodic surveys for gill browning in animals from clean areas as well as from areas proximate to real or suspected sources of contamination.

The oil levels to which the lobsters were exposed are certainly of environmental relevance, since similar or higher concentrations can be expected at oil spill sites (Boehm et al. 1978; Marchand 1978; Mackay et al. 1981). Of particular interest would be uncontrolled blowouts in the offshore or circumstances in which relatively high concentrations of hydrocarbons might persist in estuarine waters. Also, in relation to chronic inputs, it is worth noting that petroleum refinery waters may contain much higher levels of oil and grease (EPS 1974), and concentrations up to 30 mg/L (more than 30 times the total hydrocarbon concentrations used in the present study) are permissible in production waters at offshore drilling rigs in the North Sea (Schreiner 1980).

Summary

In summary, this study has delineated the effects of chronic low-level hydrocarbon exposure on a marine crustacean species by studying changes at various levels of biological organization. A suite of 56 different parameters including biochemical, physiological, and morphological indices were investigated. The relative sensitivity of biochemical–physiological parameters compared with general condition indices for assessing possible effects on animal health were well established in the present study. Although most plasma indices appeared not be significantly affected by oil exposure, the ones affected were plasma calcium, protein, lipid, amino acids, and plasma freezing-point depression. We believe there is rationale to suggest that some of these changes are of special significance in relation to animal health. For instance, a particularly dramatic effect was noted on blood hemocytes, in which lipid concentrations were reduced by one-half. Three different types of oil-induced effects were noted for the essential amino acids including (a) change in one sex only, (b) change in both sexes, and (c) interaction between sex and oil exposure. Meanwhile, alanine was the only nonessential amino acid to be affected by oil. The changes observed in plasma levels of some amino acids and amino acid analogs are of particular interest, since it is known that, in mammals, their alteration may be indicative of metabolic stress. One of the most interesting effects observed was the marked increase in gill browning that occurred in both sexes of the experimental group. Gill browning is a response that is easily measurable and would appear to have potential as a biological indicator for monitoring point sources of marine environmental pollutants such as petroleum hydrocarbons.

Acknowledgments

We are most grateful to Dr Mike Gilgan, Department of Fisheries and Oceans, Halifax, for his advice with respect to handling lobster blood and to Doug Hall, Department of Biochemistry, for assisting with amino acid analysis. We are also thankful to Madonna King and Alice Cadigan for performing many of the chemical analyses. This investigation was supported in part by grants from the Natural Sciences and Engineering Research Council of Canada to G.L.F. and R.J.T.

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Use of Variation in Biological Characters for the Classification of Anadromous Rainbow Smelt (*Osmerus mordax*) Groups[1]

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Analysis of meristics, growth patterns, and fecundity to somatic weight relationships of spawning anadromous rainbow smelt (*Osmerus mordax*) in Quebec waters demonstrated the existence of three geographical groups (Chaleur Bay, south shore of the St. Lawrence Estuary, and Saguenay Fjord). Although homing to spawning rivers does not appear to occur, the degree of heterogeneity of spawning smelt of different geographical areas is less at the extremes of their Quebec distribution. Classification of smelt from the commercial fishery catch indicates limited mixing of these groups during open-water migration. The existence of a fourth group is suggested by classification analysis of smelt caught along the lower north shore of the St. Lawrence Estuary.

FRÉCHET, A., J. J. DODSON, AND H. POWLES. 1983. Use of variation in biological characters for the classification of anadromous rainbow smelt (*Osmerus mordax*) groups. Can. J. Fish. Aquat. Sci. 40: 718 – 727.

L'analyse de variables portant sur des caractères méristiques, de croissance et de relations fécondité-poids somatique de l'éperlan (*Osmerus mordax*) anadrome du Québec pendant la période du frai, indique l'existence de trois groupes géographiques (la Baie des Chaleurs, la rive sud du fleuve Saint-Laurent et le fjord du Saguenay). Même si le phénomène du homing ne semble se produire ici, le degré d'hétérogénéité des regroupements d'éperlans d'origines géographiques différentes est moins important aux limites de leur distribution au Québec. La classification de l'éperlan de la pêche commerciale indique un faible degré de mélange de ces groupes lors de leur migration en pleine mer. Les résultats de la classification de l'éperlan capturé le long de la basse Côte-Nord de l'estuaire du Saint-Laurent suggèrent l'existence d'un quatrième groupe.

Received September 3, 1982
Accepted February 25, 1983

Reçu le 3 septembre 1982
Accepté le 25 février 1983

A fish stock has been described by Larkin (1972) as "a population of organisms which, sharing a common gene pool, is sufficiently discrete to warrant consideration as a self-perpetuating system which can be managed." This is founded on the presence of isolating mechanisms that limit gene flow. Discreteness in fish stocks is a property that results from the interaction between ecological and genetic factors. This duality has led investigators to qualify given stocks as genotypic or phenotypic (Booke 1981).

Members of the genotypic stock should conform to the Castle–Hardy–Weinberg law whereas the phenotypic stock would display a clinal pattern of biological variables (Spangler et al. 1981). Phenotypic stocks are characteristic of *r*-selected, short-lived fish, pelagic as fry, and dependent on very few age classes in the spawning stock (MacLean and Evans 1981; Ryder et al. 1981). Various processes may help to establish and maintain such stocks. Homing is one that limits gene flow. Stock discreteness can also be obtained by mutation, selection, and genetic drift (MacLean and Evans 1981).

Tagging studies (Magnin and Beaulieu 1965) in the St. Lawrence Estuary have demonstrated that seasonal open-water migrations in rainbow smelt (*Osmerus mordax*) are generally between 80 and 160 km. Marcotte and Tremblay (1948) found a north–south gradient in the vertebral counts of anadromous rainbow smelt of the Gaspé Peninsula. Homing has not been demonstrated in anadromous rainbow smelt (Magnin and Beaulieu 1965); thus, the extent of gene flow between the various spawning rivers is unknown.

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[1] Contribution to the program of GIROQ (Groupe interuniversitaire de Recherche océanographique du Québec).

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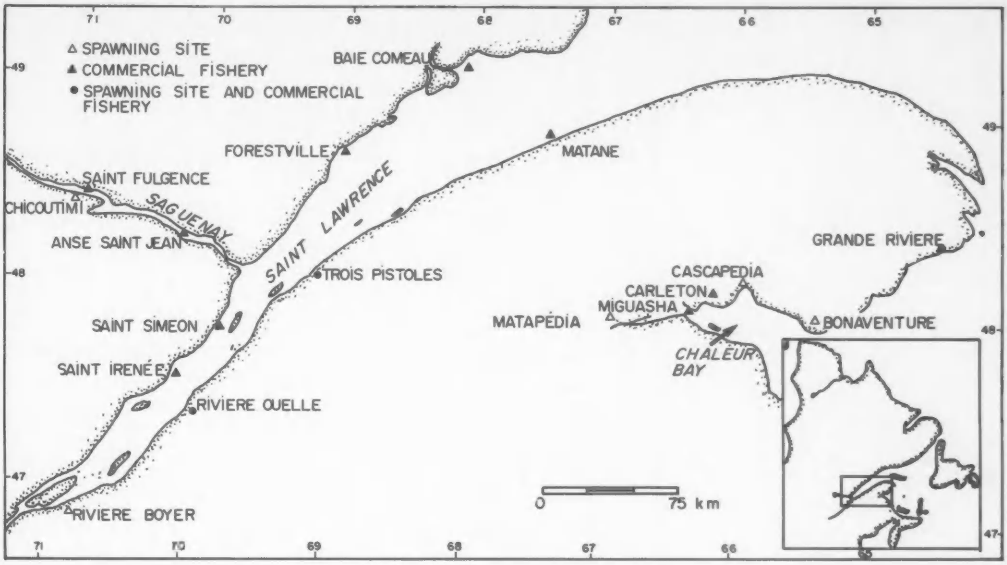
Printed in Canada (J7052)
Imprimé au Canada (J7052)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△ SPAWNING SITE
▲ COMMERCIAL FISHERY
● SPAWNING SITE AND COMMERCIAL FISHERY
BAIE COMEAU
FORESTVILLE
MATANE
SAINT FULGENCE
CHICOUTIMI
SAGUENAY
SAINT LAWRENCE
ANSE SAINT JEAN
TROIS PISTOLES
SAINT SIMEON
SAINT IRENÉE
RIVIERE OUELLE
RIVIERE BOYER
GRANDE RIVIERE
CASCAPEDIA
CARLETON
MIGUASHA
MATAPEDIA
BONAVENTURE
CHALEUR BAY
0
75 km

FIG. 1. Study areas on the Saguenay Fjord, the St. Lawrence Estuary, and Chaleur Bay.

The objective of this paper is to assess the degree of spatial integrity of smelt groups in rivers and in open waters using variation in the biological characteristics of meristics, growth, and fecundity. It is proposed that substantial differences in these characters between smelt of adjacent spawning rivers would be indicative of homing and the presence of discrete genetic stocks. Once spawning smelt populations are characterized and the extent of overlap in their biological characters is evaluated, a classification method is applied to the commercial fishery catch to assess the degree of spatial integrity of smelt stocks during their open-water migrations.

Materials and Methods

A total of 2239 anadromous spawning smelt were sampled by dipnetting during April and May, 1979 and 1980, at spawning sites in eight rivers located along the Saguenay Fjord (region I), the south shore of the St. Lawrence Estuary (region II), and Chaleur Bay (region III) (Fig. 1). A minimum of 200 fish was sampled at each spawning site. Physical characteristics of these rivers are diverse, drainage basin areas ranging from 22.7 to 79 800 km^2 and maximum flow ranging from 233 to 5830 m$^3 \cdot$s^{-1}. Another 2500 smelt were obtained from coastal commercial fisheries during the summers and winters of 1979 and 1980 (Fig. 1). Fishing gear of the commercial fishery was nonselective (box nets and weirs) in 80% of all samples and areas whereas gill nets of varying mesh sizes accounted for the remaining 20%.

All fish were frozen upon capture for subsequent analysis. Shrinkage in standard length due to freezing was compensated by applying the regression equation $y = 1.07x - 2.38$ ($R^2 = 0.986$, $n = 140$) to standard lengths measured from frozen fish (x = standard length of frozen fish, y = standard length of fresh fish).

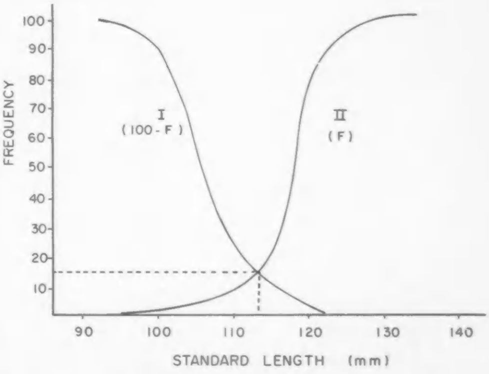

FIG. 2. Example of method for calculating the critical length interval for length-at-age values of spawning smelt.

Standard length (to the nearest millimetre), total weight (grams), gonad weight (grams), sex, and state of maturity (Nikolsky 1963) were noted for each specimen. Age was determined from scales (McKenzie 1958). Meristic counts included total left pectoral fin and anal fin rays, spines of the first left gill arch, and vertebrae (hypural excluded). Fecundity values were estimated by the dry weight method (Bagenal 1978) after treatment with Gilson's fluid and 24 h of drying at 57°C. Five samples of 100 eggs were taken from each of 208 females and weighed to the nearest 0.1 mg. The

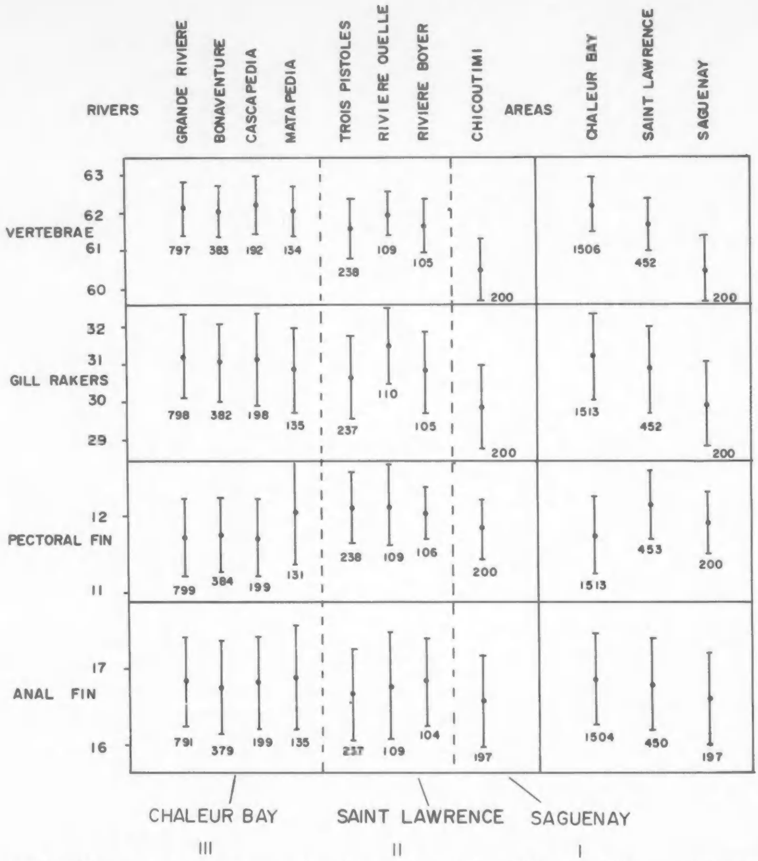

FIG. 3. Means, standard deviations, and numbers of the meristic variables recorded for spawning smelt by river and area.

TABLE 1. Univariate F value (ANOVA) of meristic values before and after pooling. $F(7, \infty, 0.001) = 3.47$; $F(2, \infty, 0.001) = 6.91$.

Character	n	Eight spawning rivers	Three areas
Vertebrae	2158	123.4	413.7
Gill raker	2165	14.0	118.0
Pectoral	2151	33.9	79.9
Anal	2166	6.0	15.7

procedure was repeated when the size of the 95% confidence interval was greater than 10% of the estimated mean fecundity. This occurred in only 25 cases.

Data Analysis

SPAWNING SITE SAMPLES

After testing interyear and between-sex differences, data were pooled for year and sex, and the variation of each of the four meristic characters between the eight spawning rivers was tested using a one-way analysis of variance (ANOVA). Significant differences were identified using the Student–Newman–Keuls (SNK) test (Sokal and Rohlf 1969). As a result of this analysis, the eight rivers were regrouped into three geographical regions, and Wilk's λ test confirmed the validity of the pooling ($P = 0.005$). Multivariate discriminant analysis was performed to identify the relative importance of the four meristic variables used in separating the three geographical regions. Mahalanobis D^2 distance (Mahalanobis 1936) was used to calculate the percentage of fish correctly assigned to their area of origin.

The statistical treatment of growth patterns involved an analysis of variance of mean length at age for each sex and age class over the eight spawning rivers. Student–Newman–Keuls tests were carried out to identify significant differences and pool samples. Sex and regional differences in growth were tested by the Hotelling T^2 test (Bernard 1981) on parameters of the von Bertalanffy growth equation

TABLE 2. Number of significant differences (SNK, $P \leq 0.05$) of four meristic variables in the eight spawning rivers.

	Area III				Area II			Area I,
	G. Rivière	Bonaventure	Cascapédia	Matapédia	T. Pistoles	R. Ouelle	R. Boyer	Chicoutimi
Chaleur Bay (III)								
G. Rivière (800)	—							
Bonaventure (384)	1	—						
Cascapédia (200)	1	0	—					
Matapédia (135)	2	1	1	—				
Saint Lawrence (II) south shore								
T. Pistoles (239)	4	3	3	3	—			
R. Ouelle (110)	2	2	2	1	2	—		
R. Boyer (106)	3	2	2	1	0	2	—	
Saguenay (I)								
Chicoutimi (200)	4	4	4	4	3	4	4	—

TABLE 3. Standardized canonical coefficients of discriminant analysis of spawning smelt.

Variable	Function 1	Function 2
Anal fin rays	0.05899	−0.04958
Pectoral fin rays	−0.18641	0.97482
Gill rakers	0.47344	0.15947
Vertebrae	0.85952	0.12634

(Abramson 1965).

As the growth of smelt was significantly different among regions and between sexes, length-at-age intervals characteristic of spawning groups were calculated using a simple method based on the cumulative frequency distribution of observed standard lengths of male and female smelt. An example, using fictitious data (Fig. 2), illustrates the method. After having calculated frequency (f), cumulative frequency (F), and the complement of cumulative frequency ($100 - F$) of the standard lengths of same-age fish of two adjacent groups (groups I and II), the cumulative frequency distribution of group II is plotted against the complement ($100 - F$) of group I. The point at which these two curves intersect (at 113 mm in this case) determines the critical standard length that distinguishes smelt of the same age and sex of the two adjacent groups. Overlaps in length frequency of smelt from adjacent geographical areas rarely exceeded 20%. The procedure is repeated to calculate the critical standard length distinguishing smelt of the same age and sex of groups II and III, thus establishing a critical length interval by age and sex for smelt from region II. The upper limit of the critical length interval for group III was based on the maximum observed standard lengths of spawning smelt captured in region III. The lower limit of the critical length interval for group I was based on the minimum observed standard lengths of spawning smelt captured in region I. Using these three critical length intervals by age and sex, smelt from the commercial fishery catch could thus be classified to a given region (I, II, or III) on the basis of their length-at-age values.

Initially, no length interval could be established for the single sample of spawning smelt of Chicoutimi (region I), as only one age class accounted for 94% of the fish sampled. This appeared to result from the temporal and spatial heterogeneity of smelt on the spawning grounds combined with the punctual nature of the sampling program. However, fish from the commercial samples at Anse St-Jean ($n = 236$) were smaller at a given age than smelt from regions II and III and displayed a wider age span than the Chicoutimi spawning samples. Therefore, the Anse St-Jean fish were considered a spawning sample, thus enabling us to calculate the critical length interval by age and sex for group I. Use of this commercial sample to replace a spawning sample is justified by the similarity of length at age 2 of the two samples and the geographical proximity of the sampling sites.

Comparison of regression lines for the fecundity to somatic weight relationship was tested by analysis of covariance (ANCOVA) (Snedecor and Cochran 1978).

TABLE 4. Classification results of discriminant analysis of meristic variables of spawning smelt.

		Predicted group membership		
Actual group	No. of cases	Chaleur Bay (%)	Saint Lawrence south shore (%)	Saguenay Fjord (%)
Chaleur Bay (III)	1477	67.0	26.8	6.2
Saint Lawrence (II) south shore	441	32.2	47.2	20.6
Saguenay Fjord (I)	197	4.6	18.3	77.2

TABLE 5. Results of SNK tests of male standard length (SL) by spawning river and age ($P = 0.05$). Nonsignificantly different means are underlined.

Age (yr)	Spawning rivers							
2	Chicoutimi	R. Ouelle	R. Boyer	Cascapédia	G. Rivière	T. Pistoles	Bonaventure	Matapédia
SL	95.07	118.96	125.71	127.77	131.30	132.44	133.29	142.71
(*n*)	(185)	(5)	(17)	(135)	(171)	(5)	(153)	(135)
3	R. Boyer	R. Ouelle	T. Pistoles	Cascapédia	Bonaventure	G. Rivière	Matapédia	
SL	145.39	145.41	145.46	152.46	162.51	162.60	171.44	
(*n*)	(70)	(77)	(59)	(20)	(114)	(153)	(45)	
4	R. Ouelle	Cascapédia	R. Boyer	T. Pistoles	Bonaventure	G. Rivière	Matapédia	
SL	165.26	166.33	168.83	168.99	179.25	179.46	185.94	
(*n*)	(12)	(3)	(3)	(13)	(12)	(17)	(3)	
5	Cascapédia	R. Boyer	T. Pistoles	G. Rivière	Bonaventure			
SL	180.59	181.78	187.44	189.58	196.03			
(*n*)	(5)	(9)	(5)	(5)	(7)			

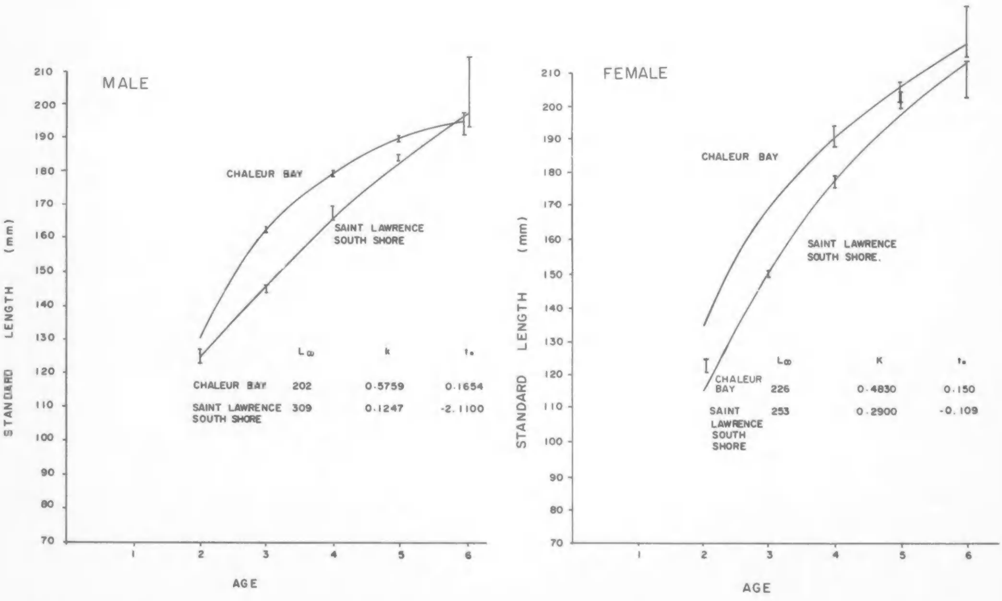

FIG. 4. von Bertalanffy growth curves for length-at-age data of spawning smelt (vertical bars represent ±1 SE of measured length).

Each method of classification (meristics, length-at-age) was then validated by the degree of overlap of accurately predicted group membership of individual fish by a simultaneous application of both methods to the spawning run samples.

COMMERCIAL FISHERY SAMPLES

To assign smelt caught in the coastal commercial fisheries to one of the spawning groups previously identified, the Mahalanobis D^2 generalized distance was calculated based on the four meristic variables. Fish from the commercial fishery were also assigned to one of the three areas of origin based on their length-at-age value.

The fecundity to somatic weight relationship could not be used for classifying fish from the commercial fishery because no fecundity count could be made between spawning runs.

TABLE 6. Results of the Hotelling T^2 test applied to von Bertalanffy growth coefficients ($L\infty$, asymptotic length; t_0, hypothetical age at zero length; k, growth coefficient; NS, not significant; $^{**}P \leqslant 0.01$; T_0^2 = critical value of T^2 for significance).

Test	T^2	T_0^2	Relative importance (critical F_0) No. 1	No. 2	No. 3
Saint Lawrence south shore, between sexes	4 093.9**	11.39	t_0 (318.75)	k (275.91)	$L\infty$ (35.28)
Chaleur Bay, between sexes	33 399 3**	11.36	$L\infty$ (2462.17)	k (395.79)	t_0 (2.47) (NS)
Males, between areas	14 193.3**	11.36	k (5327.63)	t_0 (2547.10)	$L\infty$ (759.47)
Females, between areas	3 242.9**	11.37	k (542.84)	$L\infty$ (361.84)	t_0 (71.62)

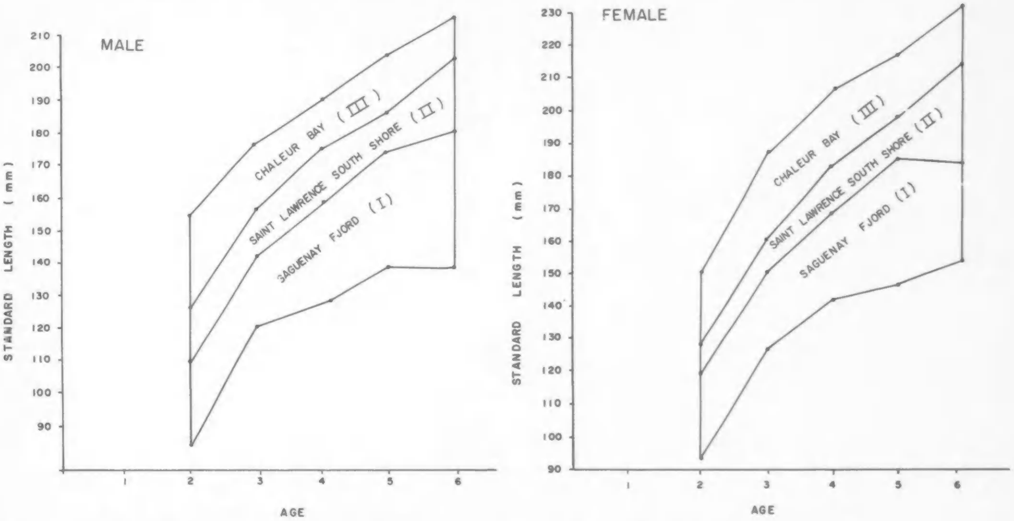

FIG. 5. Intervals of standard length-at-age based on spawning smelt for assignment of commercial fishery catch.

Results

SPAWNING RUNS

Meristics — As no significant differences were found between year and sex ($P > 0.05$), the four meristic variables recorded at each spawning river (Fig. 3) were pooled for year and sex. Each variable showed significant variance between rivers (ANOVA, $P = 0.01$) (Table 1). The a posteriori SNK test indicated an increase in the number of significant differences closely related to the geographical distance separating each spawning river (Table 2). Three geographic groups (areas, Fig. 3) were thus defined: Saguenay (I), the St. Lawrence Estuary (II), and Chaleur Bay (III). The Saguenay Fjord has the greatest number of differences in meristic counts when compared with spawning rivers of Chaleur Bay. Intermediate numbers of differences arise when one compares either area to the St. Lawrence spawning rivers. Small numbers of significant differences occur within each of the three areas.

This observation led us to pool results from the spawning rivers into three areas (I, II, and III) to obtain maximum separation of groups. The usefulness of this pooling is revealed by the increase of the F value for each meristic variable considered over the larger geographic scale (Table 1). Furthermore, a significant Wilk's λ ($P = 0.005$) confirmed the hypothesis of having effectively three smelt groups. The standardized canonical discriminant function coefficients (Table 3) of the first two functions illustrate the relative importance of the four meristic variables used. The first axis explained 89.5% of the variance between these three groups and was mainly due to the effect of vertebrae and gill raker counts.

Classification results (Table 4) based on Mahalanobis D^2 distance yield an overall value of correctly classified fish from the spawning runs of areas I, II, and III ranging from 47.2 to 77.2%.

TABLE 7. Classification results of spawning and commercial fishery catch of smelt on the basis of growth pattern.

			Predicted group membership (%)		
Actual group	No. of cases	Not assigned	Chaleur Bay	Saint Lawrence south shore	Saguenay Fjord
Chaleur Bay spawners (III)	1490	3.3	76.6	18.5	1.6
Saint Lawrence south shore spawners (II)	454	0.9	20.9	43.2	35.0
Saguenay Fjord spawners (I)	200	4.0	0.0	2.5	93.5
Commercial fisheries					
Chaleur Bay	448	11.6	54.4	28.6	5.4
Saint Lawrence south shore	410	2.2	15.6	43.2	39.0
Saint Fulgence (June 26, 1980)	92	19.6	0.0	0.0	80.4
Anse Saint Jean (Aug. 23, 1979)	52	13.5	1.9	7.7	76.9
Anse Saint Jean (May 15, 1980)	87	10.4	28.7	11.5	49.4
Anse Saint Jean (July 20, 1979)	97	36.1	0.0	1.0	62.9
Saint Irenée (Sept. 9, 1979)	145	0.0	7.5	22.8	69.7
Saint Irenée (Oct. 11, 1980)	71	2.8	4.3	43.6	49.3
Saint Siméon (July 20, 1979)	57	1.8	8.8	36.8	52.6
Saint Siméon (July 21, 1979)	61	4.9	3.3	45.9	45.9
Saint Siméon (July 22, 1979)	46	19.6	2.2	21.7	56.5
Saint Siméon (Aug. 5, 1980)	90	0.0	10.0	32.2	57.8
Forestville (Aug. 23, 1979)	141	20.6	9.9	15.6	53.9
Forestville (Aug. 3, 1980)	82	6.1	25.6	31.7	36.6
Forestville (June 12, 1979)	147	23.8	24.5	20.4	31.3
Baie Comeau (Aug. 2, 1980)	104	40.4	34.6	13.5	11.5
Baie Comeau (Aug. 29, 1979)	116	21.6	39.7	29.3	9.5

Growth patterns — Because smelt growth is different ($P \leq 0.05$) for the two sexes but not different between years ($P > 0.05$), length-at-age data were tested separately by sex. Differences were significant between the eight spawning rivers from age 2 through 5 ($P = 0.05$). The SNK test was then performed on male length-at-age to test significance of differences between spawning river samples. Results (Table 5) display a geographical gradient, with the northernmost hydrographic system having the smallest length-at-age. Samples showing no significant differences were quite close geographically and often from the same area (I, II, or III). Because a geographical gradient was established and because samples from the same area often showed nonsignificant differences, sampling sites were pooled on the

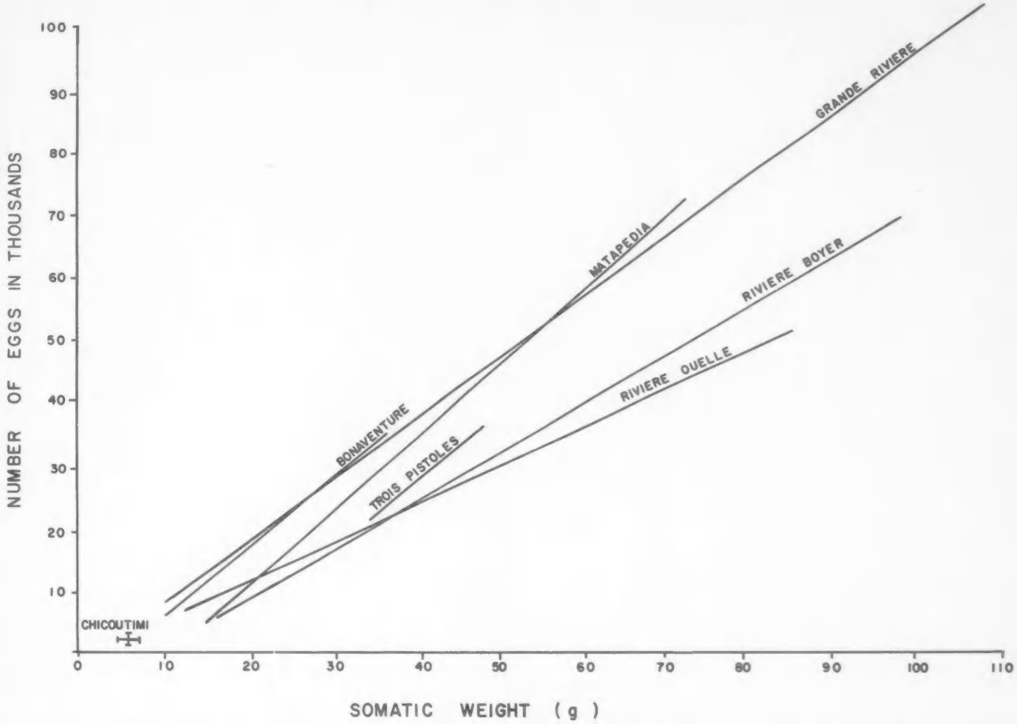

FIG. 6. Fecundity to somatic weight regressions of spawning anadromous smelt (vertical and horizontal bars for Chicoutimi sample represent ±1 SE).

TABLE 8. *F* values (ANCOVA) of the fecundity to somatic weight relationship (NA, not applicable; $^{**}P \leqslant 0.01$).

	F (elevation)	*F* (slope)
Saint Lawrence (II) south shore	8.61**	2.72
Chaleur Bay (III)	7.38**	1.24
Saint Lawrence south shore vs. Chaleur Bay	NA	34.89**

basis of their geographical distribution (I, II, or III). A similar pattern was observed for female standard length, but low numbers of females at certain spawning sites did not provide sufficient data for fine-scale comparison.

von Bertalanffy growth curves were fitted by area and sex (Fig. 4). None were fitted at the Saguenay spawning site (Chicoutimi) because the fish sampled lacked an adequate age span. The Hotelling T^2 test was then performed to compare von Bertalanffy growth parameters between sexes and areas (Table 6). All differences were significant except between sexes (t_0 parameter) in Chaleur Bay.

Before attempting to classify fish from the commercial fishery catch to a given spawning area, the application of length-at-age intervals (Fig. 5) to fish from spawning river samples was tested. Here, 1524 of 2144 (71.1%) fish could be correctly assigned to their spawning area (Table 7).

Once methods for classifying fish on the basis of meristics and growth had been tested, they were used simultaneously. Of a possible 1344 fish assigned to their area of origin by only one method, 996 (74%) fish were successfully assigned to their area of origin using both methods, thus validating the application of both methods to the commercial fishery catch.

Fecundity analysis — The fecundity to somatic weight regressions were calculated for smelt from each spawning site (Fig. 6). Simple linear regressions of fecundity on somatic weight gave better fits than regressions of transformed values (e.g. log transform). As values obtained for Chicoutimi were small (number of eggs ranging from 506 to 2862 and somatic weight from 5.8 to 7.3 g (n = 10)), no regression could be applied. Regression coefficients within a given area did not differ (ANCOVA, $P > 0.05$) (Table 8). However, the two areas (south shore of the St. Lawrence and Chaleur Bay) are significantly different (ANCOVA, $P < 0.01$) (Table 8). The regression equations were as follows: St. Lawrence south

TABLE 9. Classification results of commercial fishery based on discriminant analysis of meristic values of spawning smelt.

Actual group	No. of cases	Predicted group membership (%) Chaleur Bay	Saint Lawrence south shore	Saguenay Fjord
Chaleur Bay (III)	455	63.3	32.5	4.2
Saint Lawrence (II) south shore	451	22.6	45.9	31.5
Saguenay Fjord (I)	372	16.4	17.5	66.1
Saint Lawrence north shore				
Saint Irenée (Sept. 3, 1979)	102	6.9	18.6	74.5
Saint Irenée (Oct. 11, 1980)	97	8.2	19.6	72.2
Saint Siméon (July 20, 1979)	68	3.0	14.7	82.3
Saint Siméon (July 21, 1979)	66	3.0	7.6	89.4
Saint Siméon (July 22, 1979)	59	8.5	23.7	67.8
Saint Siméon (Aug. 5, 1980)	100	2.0	22.0	76.0
Forestville (June 12, 1979)	102	36.3	27.4	36.3
Forestville (Aug. 23, 1979)	174	35.0	41.4	23.6
Forestville (Aug. 3, 1980)	98	51.0	25.5	23.5
Baie Comeau (Aug. 29, 1979)	116	27.6	39.6	32.8
Baie Comeau (Aug. 2, 1980)	99	22.2	27.3	50.5

shore, $E = 718.71W - 3281.22$ ($R^2 = 0.760$, $N = 97$); Chaleur Bay, $E = 1061.96W - 4110.79$ ($R^2 = 0.846$, $N = 92$); where E = number of eggs and W = somatic weight.

COMMERCIAL FISHERIES

Meristics — The classification of smelt from commercial fishery samples on the basis of meristics revealed varying degrees of mixing of the three spawning groups during their open-water migrations. In the Saguenay Fjord 66.1% of the commercial catch was assigned to the Saguenay spawning group, and in Chaleur Bay 63.3% of the commercial catch was assigned to the Chaleur Bay spawning group (Table 9). Commercial catches from the south shore of the St. Lawrence exhibited the greatest degree of heterogeneity, with 45.9, 22.6, and 31.5% being assigned to the south shore, Chaleur Bay, and Saguenay Fjord spawning groups, respectively (Table 9).

As no spawning river has been identified on the north shore of the St. Lawrence, all commercial samples from this area were assigned to the already established spawning groups. Fish from the four sampling sites on the north shore fall into two groups. The first includes the St-Irenée and St-Siméon samples and are well assigned to the Saguenay group (I). A second group of samples from the downstream portion of the St. Lawrence (Forestville, Baie Comeau) are variously assigned to all spawning groups.

Growth patterns — Fish from commercial samples were assigned by the length-at-age interval method to spawning groups. The commercial fishery catch shares almost the same growth patterns as those of local spawners (Table 7). As no spawning site was found on the north shore, all commercial fishery samples were classified and two distinct groups were observed. One consisted of samples from St-Irenée and St-Siméon, which are assigned to the Saguenay group (45.9−69.7%) (Table 7). A second group of samples (Forestville and Baie Comeau) is variously assigned to all spawning stocks, with a high proportion of individuals being not assigned at all.

Discussion

Significant variations are found in three biological characters of anadromous smelt in Quebec waters. Meristics, growth patterns, and fecundity to somatic weight relations indicate significant variability between the eight spawning rivers sampled. An analysis of the pattern of variability revealed three geographical groups.

This study has demonstrated that homing to spawning rivers does not appear to occur because differences between rivers within a geographical area are small. However, the degree of heterogeneity of spawning smelt of different geographical areas is less at the extremes of their Quebec distribution. Differences between these geographical groupings are most likely due to both genetic and environmental factors. Classification of spawning smelt to an area other than the area of sampling may thus reflect either mixing of smelt from neighbouring areas or the variability of biological characters along an environmental cline, characteristic of geographical stocks.

The classification of smelt from commercial catches provides an insight into the extent of mixing over the summer season. Fish captured at the extremes of their Quebec distribution have the greatest affinity to the local spawning group. This indicates that open-water migrations are limited to the geographical area of origin and, to a lesser degree, to adjacent areas. Such local migratory movements are consistent with those recorded by tagging (Magnin and Beaulieu 1965) of smelt in the St. Lawrence Estuary, which indicated average movements from 80 to 160 km in the upstream−downstream direction. Magnin and Beaulieu (1965) also found very limited migrations between the north and south shores of the St. Lawrence Estuary.

Because no spawning river was found on the north shore of the St. Lawrence between Quebec and Baie Comeau, it is of interest to note the splitting of the four sampling sites on the north shore into two groups. Meristics as well as growth patterns assign the upstream group (St-Irenée, St-Siméon) quite well to the Saguenay area (I) whereas the downstream group (Forestville, Baie Comeau) does not relate clearly to any spawning area. This suggests the existence of an as yet unsampled spawning site along the lower north shore of the St. Lawrence Estuary. The survey for spawning rivers stopped at Franquelin, a village approximately 25 km downstream from Baie Comeau, but there may be a spawning river farther downstream that could be the source of these samples.

Fecundity observations showed a geographical gradient and record values. A maximum of 93 000 eggs was found in a 5-yr-old female at Grande-Rivière (area III). Smallest values were obtained for Chicoutimi smelt (area I). Spawning females sampled in the Saguenay exhibit growth, maturity, and fecundity similar to previously studied "stunted" freshwater smelt (Delisle 1969).

A further indication supporting the existence of at least three discrete smelt groups is the similarity between the locations of fishing areas and the identified groups. Fisheries are extremely limited between Matane and Gaspé, suggesting that smelt are relatively rare in this area and thus that estuary and Chaleur Bay populations are distinct. Finally, information on distribution of parasites (Fréchet et al. 1983) confirms the existence of groups identified here.

Acknowledgments

We thank Esther Bonneau, Alain Gagné, and Denis Croteau for their assistance in both the laboratory and the field. Special thanks to all commercial fishermen who collaborated in the sampling. Scale readings were validated by Drs D. Copeman and C. Delisle. Funding was provided by the Government of Canada, Department of Fisheries and Oceans, Quebec Region, and FCAC.

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Mark–Recapture Estimates of Harp Seal Pup (*Phoca groenlandica*) Production in the Northwest Atlantic

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Pup production of the northwest Atlantic harp seal population (*Phoca groenlandica*) was estimated using a modified Petersen model corrected for tag loss and nonreporting of recovered tags. Variance estimates were corrected for the additional sources of variation due to tag loss, reporting rate, and the estimation of the number of seals examined for tags. Experiments were conducted in March of 1978, 1979, and 1980 with 9154, 4939, and 6246 pups being tagged in each year, respectively. Estimates from short-term recoveries (within the same year) were unreliable because of failure to satisfy the assumption of uniform mixing of marked and unmarked seals. Pooled estimates from long-term recoveries (1–3 yr) were considered more reliable, with the 1978 estimate of 506 000 ± 77 000 (1 SE) and the 1979 estimate of 489 000 ± 71 000 being most reliable. Based on the results of this study and on the trend in catches of pups and immatures in West Greenland, we conclude that harp seal pup production and hence population size has probably increased since the imposition of catch quotas in 1971.

BOWEN, W. D., AND D. E. SERGEANT. 1983. Mark–recapture estimates of harp seal pup (*Phoca groenlandica*) production in the northwest Atlantic. Can. J. Fish. Aquat. Sci. 40: 728–742.

La production de chiots de la population de phoques du Groenland (*Phoca groenlandica*) de l'Atlantique nord-ouest a été estimée à l'aide d'un modèle de Petersen modifié, corrigé pour tenir compte des pertes et de non-signalements des étiquettes récupérées. Les estimations de variance ont été corrigées pour tenir compte des sources de variation supplémentaires, pertes d'étiquettes, taux de signalement et estimation du nombre de phoques examinés pour détecter la présence d'étiquettes. Les expériences ont été menées en mars 1978, 1979 et 1980, le nombre de chiots marqués chaque année étant de 9 154, 4 939 et 6 246 respectivement. On ne peut se fier aux estimations de recaptures à court terme (la même année), parce qu'elles ne satisfont pas l'hypothèse d'un mélange uniforme des phoques marqués et non marqués. On considère la mise en commun d'estimations de retours à long terme (1 à 3 ans) comme étant plus fiable, les estimations de 506 000 ± 77 000 (1 É.T.) en 1978 et de 489 000 ± 71 000 en 1979 étant les plus fiables. D'après les résultats de cette étude et la tendance des captures de chiots et de phoques immatures au Groenland occidental, nous concluons que la production de chiots de ce phoque, donc l'effectif de la population, a probablement augmenté depuis l'établissement du premier contingent en 1971.

Received September 27, 1982
Accepted March 2, 1983

Reçu le 27 septembre 1982
Accepté le 2 mars 1983

NORTHWEST Atlantic harp seals (*Phoca groenlandica*) have been hunted intensively since the early 19th century (Colman 1937). Over this period the population has been depleted several times, most recently from about 1950 to the early 1970's. A variety of past workers have concluded that by 1970 the population had been reduced by perhaps 50% of its 1952 level (Allen 1975; Lett and Benjaminsen 1977; Winters 1978; Lett et al. 1979; Beddington and Williams 1980). Estimated annual catches of harp seals during this period averaged 304 000 animals, ranging from 202 000 to 402 000 (Bowen 1982).

To halt this decline in numbers, the Canadian Government in 1971 imposed restrictions on the number of harp seals that could be killed. Since then, information on the population dynamics of harp seals has been reviewed annually and the total allowable catch has been based on population assess-

Printed in Canada (J7069)
Imprimé au Canada (J7069)

ments presented to the Northwest Atlantic Fisheries Organization (NAFO) (formerly the International Commission for Northwest Atlantic Fisheries (ICNAF)). The allowable catch of harp seals was set at 245 000 in 1971, 150 000 from 1972 to 1975, 127 000 in 1976, 170 000 in 1977, and 180 000 from 1978 to 1980. Actual catches exceeded the allowable catch in 1975 and 1976 but were less than or equal to that allowed in all other years.

Restrictions on the level of kill reduced average annual catches by 43% between 1971 and 1980. Since 1974, various workers have attempted to determine if this reduction was sufficient to halt the population decline. Trends in abundance and productivity since 1971 have been assessed using cohort analysis (Lett and Benjaminsen 1977; Lett et al. 1979; Winters 1978) and other population models (Benjaminsen and Øritsland 1975; Beddington and Williams 1980). The results of the cohort analyses all indicated that the population was recovering. However, the agreement between those studies is not surprising, as similar methods were used. Benjaminsen and Øritsland (1975) suggested that production in 1967 was larger than previously calculated and that the population was increasing under reduced catches. Using a χ^2 fitting procedure, Beddington and Williams (1980) concluded that the harp seal population had continued to decline since 1971 and that the rate of natural mortality had been underestimated in other studies. However, it now appears that the method of Beddington and Williams cannot be used to reliably estimate both M and trends in abundance using only catch-at-age data (J. R. Beddington, York University, United Kingdom, personal communication; Roff and Bowen 1983).

Direct and indirect census methods also have been used to estimate trends in abundance since 1971. Although no reliable method of estimating the number of older seals has been devised, several methods are available to estimate pup production that at least have the potential of producing reliable results. Lavigne et al. (1975), Lavigne (1976), and Lavigne et al. (1980) conducted aerial photographic surveys in 1975 and 1977 to estimate harp seal pup production in the northwest Atlantic. Both surveys suffered from technical problems and inclement weather and produced unreliable results or at best substantial underestimates of total production. Furthermore, both surveys were conducted too soon after the first quota to enable a reasonable test of the hypothesis that the population was recovering. Significant increases in numbers of recruits to the breeding population could not have been expected until 1977 (due to the 5 yr taken for females to mature on average), and by 1977 only about 50% of females from the first large quota year class (1972) would have contributed to production.

The purpose of the present study is to estimate harp seal pup production using a Petersen-type mark−recapture model. Major experiments were conducted in March of 1978, 1979, and 1980. The period of time between the first quota and these experiments is thought to have been sufficient to allow the first reasonable test of the hypothesis that pup production has increased since 1971.

Materials and Methods

SEAL BIOLOGY

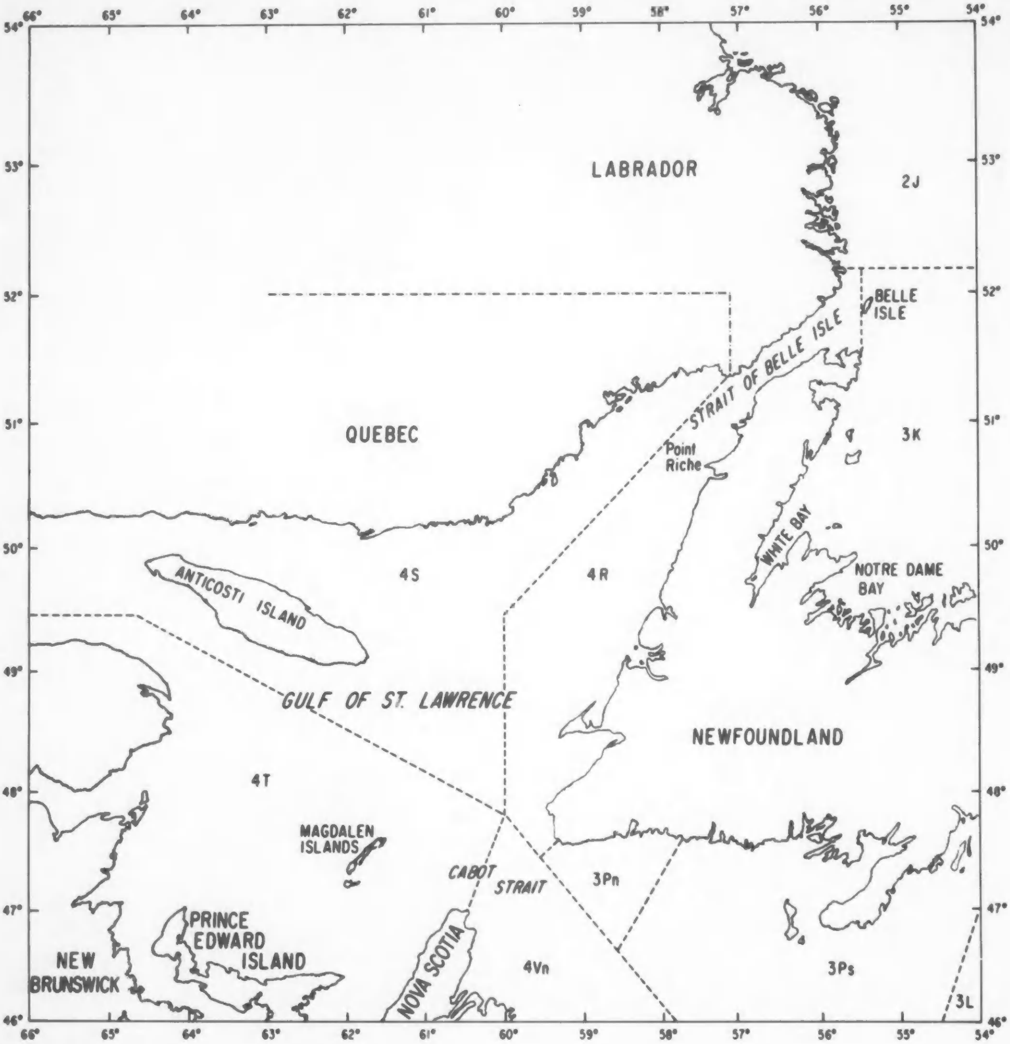
To understand the nature of the mark−recapture experiments, we must first understand the biology of harp seals and the way in which they are hunted. Harp seals are migratory marine mammals inhabiting Arctic waters in summer and waters off Newfoundland in winter. They begin their southward migration in late September, and by December the first migrants have reached northern Newfoundland. Here, the population separates; about one-third of the mature seals enter the Gulf of St. Lawrence to breed near the Magdalen Islands (known as the "Gulf" herd) and the remainder continue down the east coast of Newfoundland and eventually breed on pack ice off southern Labrador (known as the "Front" herd) (Fig. 1). During the winter most immature harp seals feed at the Front area, although some migrate into the Gulf of St. Lawrence (Sergeant 1965). Long-term tag recoveries in the mark−recapture experiments came from immatures that were shot or netted from December to May mainly at the Front.

Most pups are born over a 1-wk period beginning in late February in the Gulf and about March 8 at the Front. Newborn pups are covered with a long white fur from which they derive the name "whitecoats." These whitecoats are killed by sealers operating from large offshore vessels. Pups are nursed for about 9 d and then are abandoned by their mothers (Stewart and Lavigne 1980). Abandoned whitecoats at the Front drift southward on the ice floes to rich feeding grounds in White and Notre Dame bays (Fig. 1). During this period they begin to shed their white fur, and by about 18 d of age the neonatal coats are replaced with the spotted juvenile pelage. Moulted pups are known as "beaters." In the Gulf, whitecoats generally drift toward Cabot Strait, and as beaters they migrate north along the west coast of Newfoundland. From late March to May, beaters are shot from small boats. It is this hunt for beaters that allows the short-term recovery of tagged animals.

SAMPLING METHODS

Harp seal whitecoats (ages 2−12 d) were marked with numbered, colored Roto-tags (Dalton Supplies Ltd., Henley-on-Thames, Oxon, England). Tags were applied to the left flipper or, in the case of double-marked seals, to both hind flippers. Sex, pelage type (i.e. approximate age) (Stewart and Lavigne 1980), and tag numbers were recorded for double-marked animals.

Helicopters were used to distribute the tagging effort as widely as possible throughout whelping concentrations located in the Gulf and at the Front. Generally, in the Gulf, four people tagged seals working from a single aircraft, while at the Front, tagging was performed by six to eight people operating from one or two helicopters or, as in 1979, from helicopters and sealing vessels. Pups were tagged, as far as possible, in areas remote from sealing activity to reduce the chance of premature recovery of tags. At each tagging location on the ice, each person walked in a different direction from the helicopter and marked every whitecoat encountered until about 250 pups had been marked. This process was then repeated in another area, the direction of which was chosen at random. Brightly colored dye was used to mark the ice at locations where tagging took place. The chance of concentrating all tags in one part of the whelping area was therefore minimized.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GULF OF ST. LAWRENCE
NEWFOUNDLAND
4T
MAGDALEN ISLANDS
CABOT STRAIT
3Pn
PRINCE EDWARD ISLAND
NEW BRUNSWICK
NOVA SCOTIA
4Vn
3Ps
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FIG. 1. Place names used in the text and NAFO subareas.

To encourage the return of tags, hunters providing information on the date and location of tag recoveries were paid a reward of $10 per tag. Some hunters (generally <10%) returned tags without providing information on where and when the tag was recovered. In these cases the hunter was contacted by letter or telephone and the reward was paid when all available information about the tag had been obtained.

The experiments were originally designed to provide an estimate of production from tags recovered from beaters in April and May of the same year (short-term recoveries). However, it was soon recognized that because of nonrandom distribution of tags during this short period of hunting, more reliable results might be obtained by using longer-term recoveries from 1-, 2-, and 3-yr-olds. By this time, the animals have migrated to the Arctic and back perhaps several times, and marked animals will have mixed themselves among the general population of their age-class to a greater extent.

The Petersen method was used to estimate pup production. Let M be a sample of whitecoats marked and returned to the population. Subsequently, a second sample of n animals is taken and it is found that m are marked. Assuming that the proportion of marked in the second sample is a reasonable estimate of the unknown population proportion, then following Chapman (1951), an estimate of N is given by

$$(1) \quad N^* = \frac{(M + 1)(n + 1)}{(m + 1)} - 1.$$

A number of assumptions must hold if N^* is to be a reasonable estimate of N. These are listed as follows.

1) The population is closed, so that N is constant.
2) All animals have the same probability of being caught in the first sample.
3) Marking does not affect catchability.
4) Animals are correctly classified as marked or unmarked on recovery.
5) Animals do not lose their marks in the time between the two samples.
6) All marks are reported accurately on recovery in the second sample.
7) The second sample is a simple random sample.

The value N^* is an underestimate of N whose bias declines exponentially very rapidly as the expected number of recaptures increases. This is a negligible consideration when more than about seven animals are expected to be recaptured (Chapman 1951; Robson and Regier 1964).

An estimate of the variance of N^* is given by Seber (1973) as

$$(2) \quad \mathrm{var}(N^*) = \frac{(M + 1)(n + 1)(M - m)(n - m)}{(m + 1)^2 (m + 2)}.$$

Approximate 95% confidence limits of N^* are given by

$$(3) \quad N^* \pm 1.96 \sqrt{\mathrm{var}(N^*)}.$$

However, according to Ricker (1958), in general it is better to base confidence limits on the probability distribution of m by entering m as a Poisson variable and looking up approximate values from tables such as given in Ricker (1975, p. 343). For values of $m > 50$, approximate 95% confidence limits of m are given by

$$(4) \quad m + 1.92 \pm 1.96 \sqrt{m + 1}.$$

In the present study, both methods were tried and produced similar results. We present confidence limits based on eq 3. These limits, however, presume that all the assumptions of the statistical model are upheld. As will be shown, corrections to Chapman's estimator and associated confidence limits must be made to account for tag loss and nonreporting of recovered tags.

If double marking is carried out and some recaptures are made with only one tag retained, then Eberhardt et al. (1979) estimate the probability of loss (ℓ) as follows:

$$(5) \quad \ell = \frac{m_s}{m_s + 2m_d}$$

where m_s is the number of recaptures with only a single mark retained and m_d is the number of recaptures with both marks retained. The variance of tag loss is given by

$$(6) \quad \mathrm{var}(\ell) = \ell^2 (1 - \ell)^2 \left(\frac{1}{m_s} + \frac{1}{m_d}\right).$$

A portion of recovered tags may not be reported, which will lead to a biased estimate of N if not corrected. Rate of reporting (r) was estimated from a community survey and the variance of r was calculated following the method of Cochran (1963) for cluster samples with unequal cluster size. Let m_i be the number of tags recovered in the ith cluster (community) and let a_i be the number of tags recovered before the survey. The reporting rate is $r = \overset{n}{\Sigma} a_i / \overset{n}{\Sigma} m_i$ and the approximate variance is

$$(7) \quad \mathrm{var}(r) = \frac{(1 - f)}{n\bar{m}^2} \frac{\Sigma a_i^2 - 2r\, \Sigma a_i m_i + r^2\, \Sigma m_i^2}{n - 1}$$

where f is the sampling fraction and $\bar{m} = \Sigma m_i / n$ is the average number of tags recovered per community in the sample.

For estimates of N based on short-term recoveries, we need only correct the Chapman formula for tag loss (ℓ) and reporting rate (r), as the number of seals in the second sample is known without significant error. The number of beaters (O-group) killed in Fisheries Management unit areas (Fig. 2) between late March and May of 1978, 1979, and 1980 was obtained from the Economics Branch of the Department of Fisheries and Oceans, St. John's.

There is an additional source of error in estimates of N based on long-term recoveries. This arises because the number of seals age i in the second sample must be estimated from the total catch of animals age 1 and older. Age-specific catches of the 1978, 1979, and 1980 cohorts through 1981 were estimated by prorating the total annual catch of seals age 1 and older using age samples from the various components of the hunt collected each year during the period of tag recoveries. Total annual catches of harp seals in the Gulf and at the Front were obtained from ICNAF/NAFO statistical bulletins (Anonymous 1980a, 1980b, 1980c, 1981). Let $n_{i,t}$ be the number of seals age i killed in year t and let $C_{T,t}$ represent the total 1+ kill in year t. If a random sample $C_{s,t}$ is taken from $C_{T,t}$ for age determination, then we define $p_{i,t} = C_{i,t}/C_{s,t}$ as the proportion of animals age i in the sample. The number of seals age i in year t is then estimated as

$$(8) \quad n_{i,t} = p_{i,t} \cdot C_{T,t}$$

and the variance of $n_{i,t}$ is given by

$$(9) \quad \mathrm{var}(n_{i,t}) = \frac{C_{T,t}(C_{T,t} - C_{s,t})}{C_{s,t} - 1} p_{i,t}(1 - p_{i,t}).$$

The number of seals in the second sample for pooled estimates of long-term recoveries is $\Sigma n_{i,t+i}$ where $i = 1, 2, 3$ and $t = 1978, 1979, 1980$. The estimated variance of $\Sigma n_{i,t+i}$ is the sum of the individual binomial variances given by eq 9.

Ignoring the various values of unity, since these are really only important if quite small samples are used, Chapman's eq 1 may be rewritten to adjust for tag loss and nonreporting

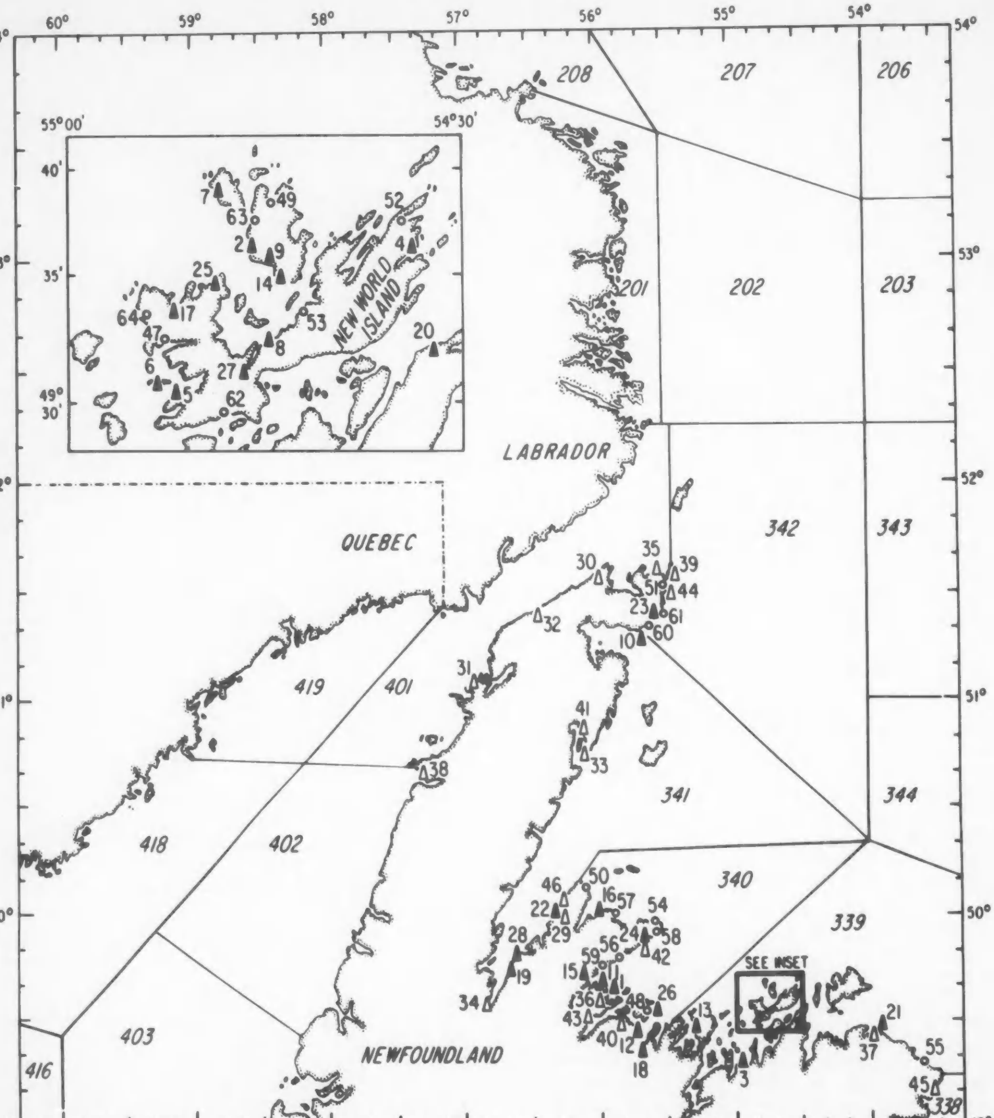
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FIG. 2. Location of communities surveyed for nonreporting in 1979 and 1980 and of Fisheries Management unit areas (e.g. 342). 1979 (△): Beachside (1), Bluff Head Cove (2), Cambellton (3), Cobbs Arm (4), Cottles Cove (5), Cottles Island (6), Crow Head (7), Fairbanks (8), Gillard's Cove (9), Goose Cove (10), Jackson's Arm (11), Leading Tickles (12), Little Harbour (13), Manuel's Cove (14), Middle Arm (15), Mings Bight (16), Morton's Harbour (17), Point Leamington (18), Purbeck's Cove (19), Port Albert (20), Ragged Point (21), Seal Cove (22), St. Anthony Bight (23), Tilt Cove (24), Tizzard's Harbour (25), Triton (26), Virgin Arm (27), Westport (28); 1980 (▲): Baie Verte (29), Boat Harbour (30), Brig Bay (31), Eddie's Cove East (32), Englee (33), Hampden (34), L'Anse Au Meadows (35), Little Bay (36), Musgrave Harbour (37), Port Saunders (38), Quirpon (39), Robert's Arm (40), Roddickton (41), Round Harbour (42), Springdale (43), St. Lunaire (44), Wesleyville (45), Wild Cove (46); both years (○): Bridgeport (47), Brighton (48),

TABLE 1. Number of whitecoats tagged in March of 1978, 1979, and 1980 in the Gulf and Front herds.

			Tagged		Total	
Year	Date	Area	Single	Double	Area	Year
1978	1–19	Gulf	4 378		4 378	
	14–19	Front	5 000		5 000	9 378
1979	2–18	Gulf	2 680		2 680	
	12–18	Front	2 464	420	2 884	5 564
1980	3–14	Gulf	2 738	894	3 632	
	14–18	Front	2 080	330	2 410	
	14–24	Strait[a]	978	227	1 205	7 247
Total			20 318	1871	22 189	22 189

[a]Strait of Belle Isle; generally considered part of the Front (Fig. 1).

of recovered tags. Using single tag recoveries only, we have

$$(10)\quad N^*_{\text{adj}} = \frac{M \sum_{i=j}^{k} n_{i,t+i}}{\sum_{i=j}^{k} m_{i,t+i}} \cdot (1-\ell) \cdot r$$

(short-term estimates: $j = 0$, $k = 0$;
long-term estimates: $j = 1$, $k = 3$).

The variance of N^* is derived on the assumption that the ratio m/n is a random variable given that n has been selected. If we remove this restriction we may consider the ratio m/n a binomial random variable where n itself is also a binomial random variable. We then have by the delta method (Kendall and Stuart 1969, p. 232):

$$(11)\quad \operatorname{var}(n/m) = \frac{\operatorname{var}(n)}{m^2} + \frac{n^2 \operatorname{var}(m)}{m^4} - \frac{2\operatorname{cov}(n,m)}{m^3}.$$

In this case the covariance term is zero, since n is chosen before m is determined and m has no influence on the selection of n. Therefore, we have

$$(12)\quad \operatorname{var}(n/m) = \frac{\operatorname{var}(n)}{m^2} + \frac{n^2 \operatorname{var}(m)}{m^4}.$$

The variance of N^* adjusted for the fact that n is an estimated value with associated sampling error is given by

$$(13)\quad \operatorname{var}(N^*_n) = M^2 \cdot \operatorname{var}(n/m) = M^2 \cdot \frac{\operatorname{var}(n)}{m^2} + \frac{n^2 \operatorname{var}(m)}{m^4}.$$

Variables N^*, ℓ, and r are independent random variables and therefore, following Goodman (1960), the approximate variance of the adjusted estimate of N is given by

$$(14)\quad \operatorname{var}(N^*_{\text{adj}}) = (N^*\ell r)^2 \left[c_v(N^*)^2 + c_v(\ell)^2 + c_v(r)^2\right]$$

TABLE 2. Number of tagged whitecoats surviving the whitecoat kill in March of 1978, 1979, and 1980.

Year	Area	No. of recaptures at whelping patch	Effective No. tagged	% effectively tagged
1978	Gulf	208	4170	94.8
	Front	16	4984	99.7
	Total	224	9154	97.6
1979	Gulf	106	2574	96.0
	Front	519	2365	82.0
	Total	625	4939	88.8
1980	Gulf	31	3601	99.1
	Front	508	1902	78.9
	Strait	462	743	61.7
	Total	1001	6246	86.2

where c_v is the coefficient of variation. The coefficient of variation of N^* for estimates using long-term recoveries is given by $c_v(N^*) = \sqrt{\operatorname{var}(N^*_n)}/N^*$. The term $(N^*\ell r)^2 [c_v(N^*)^2 c_v(\ell)^2 + c_v(N^*)^2 c_v(r)^2 + c_v(\ell)^2 c_v(r)^2 + c_v(N^*)^2 c_v(\ell)^2 c_v(r)^2]$ can be neglected.

Results

TAGGING

A total of 9378, 5564, and 7247 whitecoats were tagged in 1978, 1979, and 1980, respectively, for a grand total of 22 189 (Table 1). During the three experiments, 20 318 pups were single tagged and 1871 were double tagged.

Although an effort was made to minimize the recapture of pups before they left the whelping area, some early recaptures did occur, particularly at the Front where the sealing activity was greatest. These were subtracted from the total number tagged to determine the number effectively tagged (Table 2). In the 1978, 1979, and 1980 experiments, 9154 (97.6%), 4939 (88.8%), and 6246 (86.2%) whitecoats were effectively marked, respectively. These are maximum estimates of tag survival. In 1979, a few sealers from one vessel discarded tags on the ice rather than admit that they had killed a tagged seal, despite the fact that a ten-dollar reward was paid for tagged whitecoats. However, in general, nonreporting of recovered tags from the whitecoat hunt was negligible. Fisheries officers on each sealing vessel compiled complete lists of all tags taken by sealers to ensure prompt payment of rewards.

In 1979, whitecoats were tagged from sealing vessels and from helicopters at the Front. Vessel-based tagging had been used successfully by Norwegian scientists (B. Bergflødt, Institute of Marine Research, Bergen, Norway, personal communication), and because of the low cost involved the efficiency of the method was examined experimentally in this study. In vessel-based tagging, one or two researchers worked some distance behind the sealers from four sealing vessels and tagged the whitecoats that remained on the ice after an area had been hunted. The method of helicopter tagging was described earlier. The relative number of pups surviving to the

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Durrell (49), Fleur De Lys (50), Griquet (51), Herring Neck (52), Hillgrade (53), La Scie (54), Lumsden (55), Nipper's Harbour (56), Pacquet (57), Shoe Cove (58), Smith's Harbour (59), St. Anthony (60), St. Carols (61), Summerford (62), Twillingate (63), Whale's Gulch (64).

TABLE 3. Survival of tagged whitecoats to the end of the large vessel hunt at the Front in 1979 by method of marking.

	Tagged			Recaptured[a]		
Method	Single	Double	Total	Single	Double	Escapement[a]
Vessel	866	208	1074	273(32)	60(29)	741(69)
Helicopter	1598	212	1810	159(10)	27(13)	1624(90)
Total	2464	420	2884	432(18)	87(21)	2365(82)

[a] Percentage in parentheses.

TABLE 4. Sex ratio of double-marked harp seal pups in March of 1979 and 1980.

Year	Area	Male	Female	Total	χ^2
1979	Front	180	176	356	0.04 (ns)
1980	Front	278	262	540	0.47 (ns)
	Gulf	456	407	863	2.78 (ns)
Pooled[a]		914	845	1759	2.71 (ns)

[a] Test for heterogeneity was nonsignificant (ns): $\chi^2 = 0.58$, df = 2.

TABLE 5. Detection of harp seal tags by hunters in 1980.

Tag detected	Frequency	Percentage
Before death	31	12.8
On ice	27	11.1
In water	4	1.7
After death	212	87.3
On ice	32	13.2
In water	11	4.5
Coming aboard vessel	73	30.0
Onboard vessel	58	23.9
During sculping	34	14.0
After sculping	4	1.7
Total	243	100.1

end of whitecoat hunt by large vessels (i.e. effective number tagged) for both methods of tagging is given in Table 3. Of 1074 whitecoats tagged from vessels, 31% were recaptured as whitecoats at the whelping patch. However, only 10% of 1810 pups tagged using helicopters were recovered as whitecoats. The use of helicopters in tagging harp seal pups also resulted in more efficient use of personnel, as about 1000 seals could be tagged in 1 d under ideal conditions by a team of four from a helicopter. The same group of people could tag only 300 or 400 whitecoats from vessels, and fewer of these survived to be useful in the experiment.

VALIDITY OF MODEL ASSUMPTIONS

1) *N is constant* — For the Peterson method to provide an unbiased estimate of *N*, it is generally assumed that neither additions nor losses to the population occur between the time of the first and second sample. However, such processes may in fact occur provided they do not operate differentially on the marked and unmarked animals. In the present study, the estimate of *N* relates to the number of pups that survive the whitecoat hunt (essentially the end of March). Total production is the sum of the known whitecoat kill and the Petersen estimate of the number of beaters. By the end of March, all but a few pups will have been born, and these late births will have a negligible influence on the Petersen estimate. Natural mortality does occur, especially when considering estimates based on long-term recoveries, but as will be discussed below, there is no evidence that mortality operates differentially on marked pups.

2) *All pups have the same probability of being caught in the first sample* — Harp seal pups are easily approached and captured and were tagged without regard to sex or age except on March 12 and 13, 1979. On these days, researchers working from sealing ships selectively tagged the younger pups to increase the chance that sealers would avoid tagged animals. The sex ratio of tagged pups was estimated from the number of dougle-tagged pups. There was no significant heterogeneity in the sex ratios between years and areas and so the data were pooled. Of 1759 double-tagged pups whose sex was known, 914 (52.0%) were males and 845 (48.0%) were females. This ratio does not differ significantly from the population sex ratio of 1 : 1 (Table 4) reported by Sergeant (1966).

3) *Marking does not affect catchability* — Marked harp seals might be more conspicuous than unmarked ones and might be selected by hunters because of a ten-dollar reward for each tag. To test this, sealers who recovered one or more tags in 1980 were asked the following questions during a community survey: (1) How visible were harp seal tags? and (2) When and how did you detect the tag? There were 180 respondents. To the first question, 90.6% (163) of the sealers said that tags were easily seen and only 9.4% (17) felt that the tag was difficult to see. As to the detection of tags, most tags (87.3% of 243 recovered) were reported discovered by hunters after the seal had been killed (Table 5). Therefore, selective killing of marked pups appears unlikely.

Marking may also affect the behavior of pups making them more or less catchable or may directly result in higher mortality due to disruption of the maternal bond or infection at the tag site. A direct test of the effect of marking on the subsequent catchability of seals is not possible with present data. However, R. A. E. Stewart and S. Innes (University of Guelph, Guelph, Ont., personal communication) reported no significant difference in the amount of time harp seal mothers spent with marked (dyed) and unmarked pups and concluded that marking had no adverse effect on pup nutrition. Furthermore, we have found no evidence of chronic infection at the tag site of 19 marked harp seals recovered from several months to 4 yr after tagging, and 180 sealers questioned on this matter in 1980 reported no evidence of infection or chafing caused by the tag. We conclude that the assumption of equal catchability is unlikely to result in serious bias in our experiments.

4) *Animals are correctly classified as marked or unmarked* — This assumption was tested for marked whitecoats but not for older seals. The carcasses of 187 whitecoats were examined for tags in an area where we had earlier applied tags. No tags were found, although the interdigital web on the hind flipper of 13 pups had been cut, indicating that a tag had been removed. These data suggest that tags are not missed and therefore animals are correctly classified as marked or unmarked.

TABLE 6. Estimates of tag loss (ℓ) from pups double tagged in March of 1979 and 1980.

Area tagged	Approx. age at recovery (mo)	Retained: Both tags	Retained: Only one tag	Total	$\hat{\ell}$	SE
Front (1979)		78	12	90		
Front (1980)	1	74	9	83		
Gulf (1980)		4	0	4		
Total		156	21	177	0.0631	0.0137
Front (1979)		9	0	9		
Front (1980)	2−3	112	6	118		
Gulf (1980)		86	12	98		
Total		207	18	225	0.0417	0.0098
Pooled	1−3	363	39	402	0.0510	0.0081
Combined areas	11−15	22	2	24	0.0435	0.0307
Combined area	22−26	14	2	16	0.0667	0.0471

5) *Animals do not lose their marks* — To test this assumption, 1871 whitecoats were double-tagged in March of 1979 and 1980. Of 177 double-marked pups recaptured at approximately 1 mo of age, 156 had retained both tags and 21 retained only one tag. Over the 1st mo, then, $\ell = 0.0631 \pm 0.0137$ (mean ± SE) (Table 6). At 2−3 mo of age, $\ell = 0.0417 \pm 0.0098$ based on the recovery of 225 double-tagged pups. Estimated tag loss after approximately 1 yr (recoveries from seals 11−15 mo of age) was 0.0435 ± 0.0307 ($n = 24$) and after 2 yr was 0.0667 ± 0.0471 ($n = 16$) (Table 6). Although the estimates of tag loss after 1 and 2 yr are based on small samples, there is no evidence of increasing tag loss with time. Thus, loss of Roto-tags in harp seals occurs shortly after the tags are applied and subsequent losses are negligible. Future tag loss will most likely increase as tags may become worn or brittle and therefore more prone to failure. However, for purposes of these mark−recapture studies, which use recoveries from 2 to 36 mo of age only, these problems can be ignored. There was no significant difference in ℓ based on recoveries after 1 mo or after 2−3 mo; therefore, these data were pooled. Hence, estimates of pup production, whether based on short- or long-term recoveries, were corrected using the same value of tag loss rate ($\ell = 0.0510 \pm 0.0081$).

6) *All marks are reported on recovery* — To test this assumption we surveyed 8, 38, and 37 northern Newfoundland communities from August 28 to September 9, 1979, December 5−15, 1979, and September 2−20, 1980, respectively. The 1979 survey was conducted in two parts to see if the length of time available to return tags resulted in an increase in the reporting rate. Tags recovered during the survey were added to those returned before the survey, and reporting rate was expressed as the proportion of total tags that were returned before each survey. Only tags recovered before the survey from those households actually contacted during the survey were included in the analysis. Coastal communities from Cape Freels to Port Saunders were selected at random from communities landing 10 or more seals (Fig. 2). Surveys were conducted in northern Newfoundland because the majority of harp seals are killed in this area. In each community, we obtained a list of all individuals who had purchased a sealing licence in 1979 or 1980. Subsequently, these people were contacted by telephone to determine the following: (1) if they had hunted in 1979 or 1980, (2) if they had killed a tagged seal in 1979 or 1980, and (3) if they had returned the tag(s) for the reward. If they had not returned the tag, a researcher visited the hunter and paid him the reward. The 1979 survey sampled 18% of 6630 licenced sealers in Newfoundland and the 1980 survey sampled 27% of 5718 sealers. Licence statistics were obtained from the Northwest Atlantic Fisheries Organization (Anonymous 1980b, 1981).

There was no significant difference ($\chi^2 = 0.28$, 1 df, $P > 0.05$) between the September and December surveys in 1979 in the reporting rate of recovered tags (Table 7). Hence, an increase in the length of time available to hunters to return tags will not likely result in an increase in reporting rate. As there was no difference in reporting rate, the two 1979 surveys were pooled to give a reporting rate based on 268 recovered tags of 0.690. In the 1980 survey, a total of 876 tags were recovered for an estimated reporting rate of 0.769. There was a significant difference between the estimated reporting rate in 1979 and 1980 ($t_s = 1.77$, 1264 df, $P > 0.05$). However, we required an estimate of the mean reporting rate for the period 1978−81. Therefore, we combined the 1979 and 1980 surveys, resulting in an average reporting rate of recovered tags of 0.751 ± 0.0354.

Considerable variation exists in the rate of reporting of recovered tags by communities in different parts of northern Newfoundland. To examine this variability, communities within three Fisheries Management unit areas (Fig. 2) were grouped and a three-way G-test of independence (Sokal and

TABLE 7. Estimates of reporting rate (r) of recovered harp seal tags from surveys of Newfoundland communities in 1979 and 1980 (f = sampling fraction).

Date of survey	No. of communities[a]	f	Tags recovered[b]: Before	Tags recovered[b]: During	Total	r	SE
Aug. 28−Sept. 9, 1979	8	0.046	127	48	175	0.726	0.0692
Dec. 5−15, 1979	37	0.211	58	35	93	0.624	0.0979
Pooled, 1979	45	0.257	185	83	268	0.690	0.0561
Sept. 2−20, 1980	37	0.211	674	202	876	0.769	0.0459
Grand total	64	0.366	859	285	1144	0.751	0.0354

[a] Only communities in which one or more tags were recovered.

[b] Tags recovered before the survey are from only those households actually contacted during the survey.

TABLE 8. Variation in reporting rate (r) by year and area.

Year	No. of communities	Unit area	Tags recovered Before	During	Total	r
1979	5	342	27	20	47	0.574
	10	340	56	22	78	0.718
	10	339	91	38	129	0.705
1980	6	342	218	51	269	0.810
	13	340	131	97	228	0.574
	6	339	193	30	223	0.866

Rohlf 1969) was applied to the data. There is a significant relationship between the three factors, year, area, and reporting rate. Reporting rate was different between years, confirming the previous analysis, and was significantly different in different areas both within and between surveys (Table 8). The reporting rate of communities in area 339 did not vary between years; however, significant variation was found in areas 340 and 342. Three communities were surveyed both years in area 342, seven in area 340, and eight in area 339. The variation in reporting rate using only those communities surveyed in both years was similar to that found using all communities. The factors responsible for this variation are unclear. There is no significant correlation between the rate of reporting and the number of active sealers in a community, the number of seals killed in that community, or the total number of tags recovered. Sociological factors no doubt play an important role in determining the reporting rate of recovered tags in different communities. However, the examination of such factors is beyond the scope of this study.

7) *Second sample is a simple random sample* — In practice, it is difficult to know if random sampling has been achieved. However, Seber (1973, p. 61) notes that "$\hat{N}$ is an intuitively reasonable estimate when the sample proportion of marked in the second sample faithfully reflects the population proportion." This means that the Petersen estimate is valid even if assumption 7 is false, provided there is uniform mixing of marked and unmarked animals so that the proportion (m/N) of marked through the population is constant. To test for homogeneity in the distribution of tagged animals, we tabulated the number of marked and unmarked seals killed in various unit areas for each 2-wk period. A significant χ^2 indicates heterogeneity in the proportion of marked seals in the population and hence violation of the assumption.

In 1978 there were few recaptures of marked beaters and hence the assumption of uniform mixing or random sampling could not be tested adequately. The low number of recaptures in the Gulf appears to have been caused by the concentration of hunting effort by landsmen on an unmarked whelping patch in the northern Gulf. Available data suggest that tagged and untagged beaters were not uniformly mixed even in the southern Gulf. In NAFO subarea 4Vn (Fig. 1), 12 tagged beaters were recaptured in a catch of only 58. At the Front, tagging was concentrated on a large patch of seals located on fast ice, with the result that these marked pups were not available to the landsmen hunt until late April or early May. Again, because of the small number of recaptures, data were tabulated using the larger NAFO subareas rather than the unit areas shown in Fig. 2. In subarea 3L, 15 tags were reported in May, but no other catch is reported. This illustrates the difficulty in using these kinds of data and the possible error involved. Most likely these marked seals were killed near the boundary of subareas 3L−3K or were killed in late April in subarea 3L. In total, only 19 tagged pups were recaptured in subarea 3L and only 29 in subarea 3K in catches of 4417 and 6083, respectively. However, because of the uncertain location of capture of 15 of these 19 tag recoveries, it is not possible to place much confidence in the ratio of marked to unmarked in each area. We conclude that assumption 7 was not satisfied for short-term recoveries in 1978.

Again in 1979, few tagged beaters were recaptured in the Gulf. In unit areas 401 and 402, catch statistics for the Gulf were confounded, as they included Gulf-born pups and most likely significant numbers of pups from a southern Front whelping patch that was not tagged. Seals from the southern Front patch are believed to have penetrated into the Gulf only as far as Point Riche (A. Ollerhead, Department of Fisheries and Oceans, St. John's, Nfld., personal communication) (Fig. 2). Beater catches south of Point Riche (i.e. unit area 402) should be mainly Gulf seals; however, only three recaptures were reported from this area. Consequently, a separate estimate of Gulf pup production in 1979 is not possible using short-term recoveries.

Between March 16 and May 31, 1979, there were 74 recoveries in unit areas 339−401 inclusive of beaters marked at

TABLE 9. Test for heterogeneity in the proportion of Front-marked pups in the recaptured sample of beaters by week and area, March−May 1979 ($G = 1.4 \times 10^5$, df = 12, $P < 0.05$; M = number marked; NM = number not marked). Pooled data are bracketed.

	Unit area											
	401		342		341		340		339		Total	
Date	*M*	*NM*	*M*	*NM*	*M*	*NM*	*M*	*NM*	*M*	*NM*	*M*	*NM*
March 16−31	0	854	2	155	0	16	1	9	0	3	3	1037
April 1−15	1	1558	7	389	5	487	4	282	0	2	17	2718
April 16−30	1	1408	13	908	18	939	3	471	3	82	38	3808
May 1−15	1	136	2	1544	4	1839	3	2043	2	1625	12	7187
May 16−31	0	140	2	154	2	27	0	40	0	304	4	665
Total	3	4096	26	3150	29	3308	11	2845	5	2016	74	15415

TABLE 10. Test for heterogeneity in the proportion of Front-marked pups in the recaptured sample of beaters by week and area, March–May 1980 ($G = 6.3 \times 10^3$, df = 24, $P < 0.05$; M = number marked; NM = number not marked). Pooled data are bracketed.

	Unit area											
	401		342		341		340		339		Total	
Date	*M*	*NM*	*M*	*NM*	*M*	*NM*	*M*	*NM*	*M*	*NM*	*M*	*NM*
March 9–22	3	18	2	80	2	22	2	0	0	0	9	120
March 23–April 5	4	410	9	315	27	1245	1	691	2	875	43	3536
April 6–19	8	1298	39	2579	56	1628	22	5153	9	4806	134	15464
April 20–May 3	1	1257	13	2894	9	2016	1	1317	1	659	25	8143
May 4–17	0	387	0	206	0	23	0	69	0	0	0	685
Total	16	3370	63	6074	94	4934	26	7230	12	6340	211	27948

TABLE 11. Proportion of Gulf-tagged pups recaptured in second sample of beaters by week and area, March–May 1980 (M = number marked; NM = number not marked).

	Unit area					
	401		402		Total	
Date	*M*	*NM*	*M*	*NM*	*M*	*NM*
March 16–29	2	468	4	42	6	510
March 30–April 12	16	161	25	1360	41	1521
April 13–26	55	2199	0	1873	55	4072
April 27–May 10	1	502			1	502
May 11–24	3	6			3	6
Unknown						
April	24		20		44	
March–May	1				1	
Total	102	3336	49	3275	151	6611

the northern Front whelping concentration in March 1979 (Table 9). For the analysis of mixing, the data from certain weeks and areas were pooled to eliminate null cells, as indicated by bracketed data in Table 9. A three-way G-test showed that significant heterogeneity exists in the proportion marked by area and date of capture.

In 1980 the assumption of uniform mixing was not upheld in either the Gulf or at the Front (Tables 10 and 11). The heterogeneity in proportion marked, particularly at the Front, can be reduced but not eliminated by the deletion of certain weeks or areas from the analysis. However, it is clear that estimates of production based on these short-term recoveries could be seriously biased and hence unreliable.

The validity of assumption 7 in relation to long-term recoveries was tested by comparing the observed proportion of Gulf and Front tags recovered with that expected knowing the relative number tagged in each area. A nonsignificant χ^2 is evidence of random sampling or uniform distribution of marked animals in the population and indicates that the estimates of production are probably unbiased. Pooled recoveries of the 1978 and 1979 marked cohorts were proportional to the effective number tagged in each area (Table 12). However, this was not the case for the recoveries of the 1980 cohort at age 1. Hence, the pooled estimates of production in 1978 and 1979 are probably reliable and should be given more weight than the less reliable 1980 estimate. It should be noted that the 1980 estimate will likely improve over the next year or two as sample size increases.

ESTIMATES OF PUP PRODUCTION

Short-term recoveries — The Petersen method is quite "robust" to various violations of the assumptions (Seber

TABLE 12. Tag recoveries from the 1978–80 marked cohorts in January–May of 1979–81 in NAFO subareas 2, 3, and 4 (G = Gulf = subarea 4; F = Front = subareas 2 and 3; T = total). $^{***}P < 0.001$.

Tagged		Recovered									Goodness of fit test		
		1979			1980			1981					
Year	Location	G	F	T	G	F	T	G	F	T	*O*	*E*	χ^2
1978	Gulf	21	69	90	19	44	63	15	10	25	178	161	
	Front	17	107	124	7	35	42	5	5	10	176	193	3.29 (ns)
1979	Gulf				23	51	76	2	12	14	90	82	
	Front				1	41	42	12	12	24	66	74	1.45 (ns)[a]
1980	Gulf							12	37	49	49	37	
	Front							3	12	15	15	27	8.46***[a]

[a] With Yates' correction for continuity.

TABLE 13. Estimates of harp seal pup production in the northwest Atlantic in 1978, 1979, and 1980 from short-term recoveries (M = number marked; m = number of marked beaters recovered in second sample; n = number of beaters examined for marks in second sample; N^*_{adj} = pup survivors of large vessel hunt, adjusted for tag loss and reporting rate; k = kill of pups by large vessels; N^*_T = total pup production).

Cohort	M	m	N	N^*_{adj}	k	N^*_T
1978	9154	79	26 318	2.17×10^6	72 021	2.245×10^6
1979	4606	100	17 852	586 026	112 064	698 090
1980	4884	519	31 061	208 320	100 895	309 215

1973). The heterogeneity of tag returns from beaters casts doubt on the reliability of short-term estimates and also makes the usual variance estimate doubtful. However, because these studies were predicated initially on the recovery of tags from beaters, the results of these calculations are included.

Estimates of pup production in 1978, 1979, and 1980 from short-term recoveries are given in Table 13. The estimates vary considerably between years from over 2×10^6 to about 300 000. The 1978 and 1979 estimates are clearly high and cannot be considered reasonable given the known history of the population over the past 20–30 yr. The 1980 short-term estimate falls within the 95% confidence limits of estimated production from long-term recoveries of the 1980 cohort.

Long-term recoveries — Before it is possible to estimate pup production from long-term tag recoveries, we must first estimate the number of 1+ seals of the appropriate age to be used in eq 10. The total 1+ catches from 1978 to 1981 and proportion of 1- to 3-yr-olds in these catches by hunting component and year are given in Table 14. From these data, the number at age in the second sample for the 1977–80 cohorts was calculated with corresponding standard errors (Table 15).

The 1978 estimate of production is based on 3 yr of recoveries (ages 1–3), the 1979 estimate on 2 yr, and the 1980 estimate on 1 yr (Table 16). Total production of the northwest Atlantic population in 1978, 1979, and 1980 is estimated to be 506 000 (rounded to the nearest thousand) ± 77 000 (1 SE), 489 000 ± 71 000, and 450 000 ± 81 000, respectively (Table 16). The squared coefficients of variation of N^*, ℓ, and r used in the calculation of var(N^*_{adj}) are given in Table 17.

A small number of whitecoats (n = 1230) was also tagged in the Gulf in 1977. Although not intended for use in population estimation, it is interesting to examine the returns from this tagged cohort as possibly providing another independent estimate. The pooled estimate of 1977 pup production, based on recoveries from 1978 to 1980, is 318 000 ± 49 000 (Table 16). The 95% confidence limits of the 1977 estimates do not include the mean of the 1978–80 estimates of production and overlap only slightly with the lower 95% confidence limits of these estimates.

The reason for this difference is not completely understood, but it appears to be related, in part, to the fact that only in 1977 were seals tagged in the Gulf alone, resulting in inadequate mixing of marked and unmarked animals. To test this, we recalculated the pooled estimates of 1978, 1979, and 1980 production using only recoveries from Gulf-tagged pups and then compared the results with estimates using returns from seals tagged in both areas. In each case the estimates using only Gulf tags were less than those using recoveries from seals tagged in both areas: 9.7% less in 1978, 10.5% less in 1979, and 24.9% less in 1980.

To the extent that it has been possible to test the assumptions of the model, we conclude that the 1978 and 1979 estimates from long-term recoveries are most reliable and are probably not significantly biased. The 1980 estimate, although not differing from 1978 and 1979 values, must be given less weight because of the violation of the assumption of random sampling. Estimates of production in 1978, 1979, and 1980 using tags recovered from beaters are not considered to be reliable.

Discussion

Ecologists have been using mark–recapture methods to estimate population size since the initial work by Lincoln (1930). Although such studies of population numbers of whales have generally been unproductive, those of population numbers of seals have provided some useful results. Mattlin (1978) used the Petersen method to estimate pup produc-

TABLE 14. Data used to calculate the number of recaptures in the second sample (n) for long-term production estimates. Sample size in parentheses represents the number of seals whose ages were determined from each component of the kill.

				Proportion in samples								
	1+ catches (C_T)			Age 1			Age 2			Age 3		
Year	Large vessels	Small vessels	Nets	Large vessels	Small vessels	Nets	Large vessels	Small vessels	Nets	Large vessels	Small vessels	Nets
1978	3525	32 944	9066	0.1474 (875)	0.4310 (529)	0.1252 (1366)						
1979	1011	23 360	4274	0.3078 (562)	0.5539 (204)	0.1426 (1122)	0.1512 (562)	0.2108 (204)	0.1925 (1112)			
1980	1563	28 794	6974	0.3078 (562)	0.4619 (827)	0.0656 (671)	0.1512 (562)	0.2019 (827)	0.0805 (671)	0.0907 (562)	0.1112 (827)	0.1520 (671)
1981	1211	14 330	8293	0.3255 (212)	0.3063 (284)	0.0279 (1067)	0.1368 (212)	0.1373 (284)	0.0910 (1067)	0.1321 (212)	0.1338 (284)	0.1186 (1067)

TABLE 15. Numbers at age in the second sample of the 1978–80 cohorts (age in parentheses).

	Year of recapture					
Cohort	1978	1979	1980	1981	Σn_i	SE
1977	15 853(1)	5 899(2)	4 404(3)		26 156	1026
1978		13 859(1)	6 612(2)	3061(3)	23 532	955
1979			14 239(1)	2888(2)	17 127	580
1980				5014(1)	5 014	392

tion of the New Zealand fur seal (*Arctocephalus forsteri*). Marking of northern fur seal pups (*Callorhinus ursinus*) to determine population size was initiated in 1947 (Kenyon et al. 1954). Chapman (1964) found estimates after 1952 to be biased due to mortality associated with tagging. A more reliable method was subsequently developed that involved random allocation of marks and sampling locations in the same season (Chapman and Johnson 1968). Petersen estimates of abundance have also been calculated for leopard seals (*Hydrurga leptonyx*) (Brown 1957), grey seals (*Halichoerus grypus*) (Hickling 1959; Lockie 1962), and fur seal pups (*Arctocephalus gazella*) in South Georgia (Payne 1977).

Early marking studies of harp seals provided information mainly on distribution and migration (Sergeant 1965) but also have been used to validate age determination methods (Bowen et al. unpublished data). Between 1949 and 1980, 42 522 harp seals were tagged and/or branded in the northwest Atlantic by Canadian and Norwegian investigators (Table 18). Marking of harp seal pups to determine population size was initiated in 1964 (Sergeant 1975). Between 1964 and 1970, Sergeant conducted four experiments, three in the Gulf using helicopters and one at the Front using an icebreaker, tagging between 2000 and 3500 young each time. Estimates of production were based on recoveries of whitecoats in whelping concentrations, and nonrandom sampling was probably a problem except in 1964 in the Gulf. In that year, seals were tagged and recaptured by helicopter and results may have been reasonably reliable. However, returns between ships were heterogeneous and therefore most of these previous estimates were unreliable.

The Petersen method is the simplest estimator of population size using marked individuals, but it is also relatively robust. The use of the Petersen method in this study was dictated by the biology of harp seals. Harp seal pups are born over a short period of time allowing only a single marking. Further, marked seals are recaptured during the commercial hunt, thus precluding multiple recaptures of the same individuals and hence the use of more sophisticated methods.

Estimators that have been developed are based on various assumptions, with that of equal catchability (random sampling) being extremely important. It is known, however, that these assumptions are often not satisfied in practice, resulting in unreliable estimates. In the present study, the assumptions that tags are not lost and that all tags are reported upon recovery are clearly violated; however, we have empirically estimated the bias resulting from these violations and have corrected the estimates accordingly. The critical assumption of random sampling is more difficult. It is clear from the analysis of short-term recoveries that in most cases the assumption of random sampling (or uniform mixing of marked and unmarked) is not upheld. Hence, estimates of population size based on these data could be seriously biased.

It is more difficult to test this assumption with long-term tag recoveries because it is not possible (without introducing significant additional error into the analysis) to examine the distribution of the proportion marked in the recaptured sample by area and time. Instead, we have tested to see if the number of recoveries of Gulf- and Front-tagged pups is proportional to the number tagged in each area. Recoveries from the 1978 and 1979 experiments were proportional to the number marked in each area, thus supporting the hypothesis of random sampling. This was not the case for returns from the 1980 experiment. Hence, there is doubt as to the reliability of this estimate based on only a single year's recoveries.

In practice, some departure from model assumptions is inevitable, even if tests of these assumptions fail to find violations. It is of interest then to know the consequences of such departures on the performance of the population size estimator. Two approaches have been used: simulation studies (Manly 1970; Carothers 1973; Ettershank and Ettershank 1973; Roff 1973; Bohlin and Sundström 1977; Zarnoch and Burkhart 1980) and field studies on populations of known size (Schumacher and Eschmeyer 1943; Krumholz 1944; Fredin 1950; Buck and Thoits 1965; Edwards and Eberhardt 1967; Eisenberg 1972; Mares et al. 1981). The results of these field studies suggest that overestimates based on the Petersen method tend to be uncommon and small, whereas underestimates tend to be frequent and large (see review by Buck and Thoits 1965; Eisenberg 1972; Mares et al. 1981).

Unequal catchability may arise because of what Cormack (1966) referred to as (1) heterogeneity: all animals do not have the same catchability because of inherent behavioral

TABLE 16. Estimates of pup production in 1977–80 using pooled recoveries from 1978 to 1981 for each cohort (M = number single tagged; m = number of single tags returned in second sample; n = number of 1+ seals examined for tags; N^*_{adj} = estimate of number of beaters corrected for tag loss and nonreporting; k = kill of pups during large vessel hunt in March; N^*_T = estimated total northwest Atlantic production; SE = standard error corrected for the variance of tag loss, reporting rate, and estimation of n; CL = confidence limit).

Cohort	M	m	n	N^*_{adj}	k	N^*_T	SE	95% CL	
1977	1230	93	26 156	246 547	71 616	318 163	49 124	221 879	414 446
1978	9154	354	23 532	433 683	72 021	505 704	77 337	354 123	657 285
1979	4606	149	17 127	377 333	112 064	489 397	70 794	350 640	628 153
1980	4884	50	5 014	349 057	100 895	449 952	80 586	292 004	607 901

TABLE 17. Components of total variance var(N^*_{adj}) estimates for pup production in 1977–80.

Year	Variance component		
	$c_V(N^*)^2$	$c_V(\ell)^2$	$c_V(r)^2$
1977	0.0123	0.0252	0.0022
1978	0.0044	0.0252	0.0022
1979	0.0078	0.0252	0.0022
1980	0.0259	0.0252	0.0022

TABLE 18. Summary of the number of harp seals marked in the northwest Atlantic, 1949–80.

Year	Area	Canada			Norway, pups	Total
		Pups	1+	Total		
1949	Front				95	95
1950	Front	59		59		59
	Gulf				125	125
1951	Front	178		178	29	207
1952	Front	371		371	68	439
	Gulf	203		203		203
1953	Front	304		304		304
1954	Gulf	116		116		116
1964[a]	Gulf	2791		2791		2791
1966[a]	Front	3581		3581		3581
	Gulf	1345		1345		1345
1968[a]	Gulf	2219		2219		2219
1969	Front				12	12
	Gulf	1556		1556		1556
1970[a]	Front				47	47
	Gulf	1966		1966		1966
1971	Front				68	68
1972	Front				61	61
	Gulf	525[b]	6[b]	531		531
1973	Front	934	214[b]	1148		1148
1974	Gulf	391[b]	3[b]	394		394
1975	Front				38	38
	Gulf	910[c]		910		910
1976	Front				301	301
	Gulf	566	5	571		571
1977	Gulf	1230		1230		1230
1978[a]	Front	5000		5000		5000
	Gulf	4378		4378		4378
1979[a]	Front	2884	12	2896		2896
	Gulf	2680		2680		2680
1980[a]	Front	3615		3615		3615
	Gulf	3632		3632		3632
Total	Front	16926	226	17164	719	17883
	Gulf	24508	14	24526	125	24651
	Combined	41434	240	41690	844	42534

[a] Mark–recapture experiments.
[b] Branded.
[c] Branded and tagged.

differences, or (2) contagion: capture at one time (i.e. tagging) affects the probability of future capture (i.e. learning effects). Both may be involved in a particular circumstance. With heterogeneity, the more catchable animals tend to be captured and marked, resulting in marked animals being more catchable on average then unmarked animals. Heterogeneity always leads to underestimation of population size. With contagion, over- or underestimation is possible depending on the form of the behavior response.

In the present study, it is clear that there is no difference in vulnerability of newborn harp seal pups to marking. Harp seals are recaptured by shooting, a completely different method of capture from that used in tagging, and hence previous capture should not affect the chance of future capture. Furthermore, handling of whitecoats has no demonstrable effect on the mother–pup bond or size at weaning (R. E. A. Stewart, University of Guelph, Guelph, Ont., personal communication). We conclude that the assumption of equal catchability is unlikely to be a serious source of bias in the present estimates of harp seal pup production.

Another potential source of variation in the estimates of production comes from the estimation of the catch of 1-, 2-, and 3-yr-olds (*n* in eq 1). There are two sources of this variation, sampling error and error in age determination. Preliminary studies suggest that age determination is unlikely to be a significant source of variation in estimates based on animals ≤ 3 yr of age (Doubleday and Bowen 1980; Bowen et al. unpublished data). Estimates of sampling error are based on reasonably large samples (Table 14) from each component of the kill in each year. Therefore, it is unlikely that in estimating *n* we have introduced significant bias.

The greatest potential source of bias in the present study relates to the degree of mixing of marked seals and hence the extent to which random sampling has been achieved. Serious departure from this assumption may result in either over- or underestimation. However, the pooled estimates of each experiment are approximately the same. It seems unlikely that a serious departure from random sampling would result in consistent estimates, from year to year, particularly in view of the marked variation observed in short-term estimates.

Our estimates of pup production of 506 000 ± 77 000 and 489 000 ± 71 000 in 1978 and 1979, respectively, are higher than the Lavigne et al. (1980) 1977 estimate of about 250 000 based on the results of an aerial survey. However, direct comparison of these estimates is difficult for several reasons. The first is that the 1977 aerial survey was incomplete, with only the Front whelping concentrations considered to have been adequately sampled. The second is that even here it was assumed that all pups had been born at the time the survey was conducted on March 14 and 15. However, this assumption was not tested and we simply do not know what proportion of the pups had been born. Finally, a correction of 10% was made to account for those pups that were likely hidden from the camera under rafted ice. Again, we are given no idea of how variable this quantity might be. Taken together, Front production might easily have been 20% higher.

As there was no estimate of Gulf production in 1977, Lavigne et al. (1980) estimated total production as the sum of the 1977 Front estimate (203 675) plus the estimate of Gulf production in 1975 (46 300) (Lavigne 1976) from aerial photography. This assumed that the same proportion of females in the population gave birth in the Gulf in 1977 as in 1975. However, estimates of this proportion, although crude, show considerable variation, ranging from 0.32 in 1960 to 0.47 in 1959 (Sergeant and Fisher 1960; Sergeant 1971). Using these proportions and the mean and upper 95% confidence limit on the mean Front production from Lavigne et al. (1980), total production in 1977 could have ranged from 269 000 to 423 000 pups, and from the previous discussion above was likely even higher. Add to this perhaps 5−10% for an increase in production between 1977 and say 1979 and the difference in the estimates from the 1977 aerial survey and the present mark−recapture experiments is no longer apparent.

Which of these two methods can in practice provide the more reliable estimate of the number of harp seal pups born in the northwest Atlantic remains equivocal. We conclude that of the available recent estimates, those from mark−recapture are probably most reliable. The present mark−recapture estimates indicate that pup production in the late 1970's was about 500 000. Using a method known as the survival index, Winters (1978) estimated that production between 1967 and 1971 (the 1st yr of the quota) ranged between 349 000 and 372 000 pups. Roff and Bowen (1983) estimated 1967 production to be about 362 000, with 95% confidence limits of 333 000−410 000, using a new method based on ratio of survivors in adjacent cohorts. Although these data indicate that the population has increased in size, it is not possible to state that this increase is statistically significant with the existing information. One reason for this is that different methods were used to estimate production in 1967 and the late 1970's. Both methods are subject to unknown biases, which may be in opposite directions. Given this, we did not consider it appropriate to conduct a statistical test.

Further evidence of a population increase since 1971 comes from catches of harp seals in West Greenland. The catch of harp seals fell from an average of 17 238 between 1952 and 1955 to an average of 6781 between 1965 and 1969. From 1970 to 1975, catches remained at the same level as in the previous 5 yr, an average of 6766. However, catches increased during the period 1976−79 to an average of 9588, approaching the average kills during the early 1960's (Bowen 1982). Major changes in hunting effort have apparently not occurred in this hunt, so that changes in the level of kill likely reflect the relative abundance of harp seals rather well (F. Kapel, Greenland Fisheries Investigation, Copenhagen, Denmark, personal communication). Thus, both lines of evidence point to an increase in harp seal pup production since 1971.

Finally, it must be emphasized that the present estimates of production are reliable only to the extent that the assumptions of the Petersen method are upheld. We have attempted to test the more important assumptions and to correct for departures to minimize bias. However, some assumptions have not or cannot be adequately tested to ensure that no bias exists. Therefore, it is important that further studies be conducted to monitor trends in pup production and that as many independent methods as possible be used.

Acknowledgments

We are grateful to G. Atkinson, D. Barrett, G. Badcock, B. Beck, B. Bergflødt, P. Brodie, J. Chadwick, E. Collins, P. Collins, H. Compton, T. Curran Jr., S. Dudka, L. Fudge, A. Giovanini, R. Greendale, K. Hay, W. Hoek, D. Hope, D. Martin, D. McLennon, A. Ollerhead, H. Oak, W. Penney, B. Petalas, B. Samson, W. Samson, G. Sleno, W. Squires, G. Traverse, M. Veitch, and G. Winters for assistance with the field work. T. Donahue and L. Feltham provided harp seal catch statistics and lists of sealers by community. D. Stansbury assisted in the computer analysis of tag returns and W. Hoek, D. Gauthier, D. Martin, and W. Penney compiled recovery data. M. Johnson, B. Nygaard, and A. Shaw kindly provided vessel accommodation for tagging personnel in 1979. We are grateful to the captain and crew of the C.S.S. *Hudson* for their help and hospitality in 1979 and 1980. We are grateful to the following helicopter pilots: G. (Scotty) Aldie, D. Caldwell, Y. Cote, K. Eytcheson, D. Kurk, P. Patterson, D. Venturi, L. Ward, and M. Watt. We thank R. Misra for advice on the estimation of the adjusted variance of *N*. W. Doubleday, D. Roff, K. Hay, J. Rice, and G. Winters provided valuable comments on the manuscript. We are also grateful to L. Eberhardt and R. Mohn for their thorough review of the paper.

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Structure−Toxicity Relationships for the Fathead Minnow, *Pimephales promelas*: Narcotic Industrial Chemicals[1]

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Narcosis is a reversible state of arrested activity of protoplasmic structures caused by a wide variety of organic chemicals. This nonspecific mode of toxic action was found predominant in acute toxicity studies of industrial chemicals and fish. This paper presents 96-h LC50 values for 65 industrial chemicals including alcohols, ketones, ethers, alkyl halides, and substituted benzenes. The common mode of action permitted the development of a structure−toxicity relationship as follows: $\log \mathrm{LC50} = -0.94 \log P + 0.94 \log (0.000068P + 1) - 1.25$ where P is the *n*-octanol/water partition coefficient. The data show that the toxicity of the chemicals to fish is directly comparable with the toxicity in mammals when expressed as chemical activity.

VEITH, G. D., D. J. CALL, AND L. T. BROOKE. 1983. Structure−toxicity relationships for the fathead minnow, *Pimephales promelas*: narcotic industrial chemicals. Can. J. Fish. Aquat. Sci. 40: 743−748.

La narcose peut être définie comme une condition réversible d'arrêt d'activité de structures protoplasmiques produite par une grande variété de substances chimiques organiques. On constate que ce mode non spécifique d'action toxique est prédominant dans des expériences de toxicité aiguë de substances chimiques industrielles menées sur des poissons. Nous présentons ici les valeurs de la CL50 après 96 h de 65 de ces substances, y compris alcools, cétones, éthers, halogénures d'alkyl et benzènes substitués. Le résultat de ce mode d'action commun est le développement d'une relation structure−toxicité comme suit : $\log \mathrm{CL50} = -0{,}94 \log P + 0{,}94 \log (0{,}000068P + 1) - 1{,}25$ où P est le coefficient de partition. Ces données démontrent que la toxicité des substances chimiques chez les poissons se compare directement à celle qu'on observe chez les mammifères quand on l'exprime comme activité chimique.

Received July 15, 1982
Accepted February 23, 1983

Reçu le 15 juillet 1982
Accepté le 23 février 1983

THE inventory of industrial chemicals in the United States consists of approximately 54 000 entries with more than 30 000 chemicals, and the inventory is continuously expanding. Effective hazard assessment methods must rapidly screen chemicals for their toxicity to allow testing resources to focus on the more potentially hazardous chemicals. One screening technique is to predict the toxicity of chemicals from their physicochemical properties. Relationships between toxicity and these properties must be developed around the various types of pharmacologic effects (Veith and Konasewich 1975). From acute toxicity tests conducted at our laboratory, it was apparent that a high percentage of industrial organic chemicals produced a narcotic effect in fish. Because of the large proportion of chemicals eliciting this response and the potential application of a screening technique for new chemicals, development of a prediction model for narcotic chemicals in fish received high priority.

Comprehensive reviews (Albert 1965; Crisp et al. 1967; Kaufman 1977; Roth 1980) have shown narcosis to be a nonspecific reversible physiological effect independent of chemical structure. Ferguson (1939) proposed that with narcosis, an equilibrium exists between the organism and the external phase. The physiological effect is then related to external concentration. The physical nature of narcosis leads to effects that are chemically nonspecific, as evidenced by the narcotic action of a variety of substances. Gases (Hesser et al. 1978),

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[1] This paper is a project of the EPA Structure−Activity Research Program.

Printed in Canada (J7007)
Imprimé au Canada (J7007)

aliphatic and aromatic hydrocarbons, chlorinated hydrocarbons, alcohols, ethers, ketones, aldehydes, weak acids and bases, and some aliphatic nitrocompounds (Albert 1965; Crisp et al. 1967; Gero 1965; Roth 1980) all exhibited narcotic action.

Ferguson (1939) and Mullins (1954) proposed that the chemical thermodynamic activities of various narcotics in blood are approximately equal at the point of equal physiological effects. Some of the deviations from equality may be accounted for by variations in molecular volume. Chemical activity in blood is related to the concentration of chemical in blood through the activity coefficient.

From mammalian studies (Ferguson 1939; Mullins 1954), the chemical activity needed to produce narcosis for volatile chemicals is estimated from the partial pressure to be 0.02 using the expression

$$A_{\text{nar}} \approx P_{\text{nar}}/P^{0}$$

where A_{nar} is the chemical activity of the compound at narcotic concentration, P_{nar} is the partial pressure of the compound at narcotic concentration, and P^{0} is the partial pressure for the pure chemical. Parameter A_{nar} is not easily determined for nonvolatile chemicals in mammalian tests because of the difficulty in estimating the activity coefficient. However, for aquatic studies, A_{nar} of nonvolatile chemicals can be estimated from the expression

$$A_{\text{nar}} \approx C_{\text{nar}}/C_{s}$$

where C_{nar} is the concentration of the chemical in water producing narcosis (after LC50) and C_s is the water solubility of the chemical. The estimation is valid if the thermodynamic activity of the chemical in water is equal or directly related to the thermodynamic activity of the chemical in the blood of the test organisms; i.e., the chemical in blood is at equilibrium with that in water. Under these conditions the acute toxicity of narcotic chemicals to fish can be expected to be directly related to the water solubility of the chemicals. The inverse relationship between water solubility and the *n*-octanol/water partition coefficient (log *P*) further suggests that acute toxicity may be related to log *P*.

Crisp et al. (1967) reported regular relationships between the toxicity of narcotics to barnacle larvae and carbon chain length. However, the variation of toxicity with carbon number of branched chemicals was irregular. Relationships between toxicity and molar volume were quite different for different classes of chemicals.

Konemann (1981) exposed guppies (*Poecilia reticulata*) to 50 anaesthetizing industrial pollutants for 7–14 d and obtained the following linear relationship between *n*-octanol/water partition coefficient (log *P*) and toxicity:

$$\log 1/\text{LC50} = 0.871 \log P - 4.87$$

where $r = 0.988$. The linear relationship did not improve with the introduction of a squared term in the equation, although it was stated that a deviation from linearity could be expected for chemicals with log *P* values greater than 6 due to a deviation from linearity for bioaccumulation with such hydrophobic compounds.

The present study was conducted to further examine the relationship between log *P* and toxicity. Because computer programs to automatically calculate the log *P* of chemicals directly from structure will soon be available, relating acute toxicity to log *P* will provide a predictive method to screen new industrial chemicals.

Experimental Procedures

Chemicals for this study were selected from the Toxic Substances Control Act industrial inventory. The 96-h LC50 concentrations of the chemicals were determined for fathead minnows (*Pimephales promelas*) from the Environmental Research Laboratory-Duluth culture using flow-through proportional diluters (Mount and Brungs 1967) and modified Benoit continuous-flow minidiluter systems. One compound, 3-furanmethanol, was run statically due to its high cost. Lake Superior water maintained at 25 ± 1°C was used in all tests. Routine measures of hardness (ethylenediaminetetraacetic acid) and total alkalinity of test water yielded mean values of 45.5 and 42.2 mg/L as $CaCO_3$, respectively. The arithmetic mean of the pH was 7.5 and dissolved oxygen was always greater than 60% of saturation.

Twenty to twenty-five 30-d-old fish each weighing approximately 0.12 g were randomly divided amongst the test tanks (control and five different concentrations each in duplicate for proportional diluter systems and singly for continuous-flow minidiluter systems). Fish were not fed during the test. Deaths were recorded after 1, 3, 6, 12, 24, 48, 72, and 96 h, and the median lethal concentration (LC50) was computed using the trimmed Spearman–Karber method (Hamilton et al. 1977) on a PDP-11/70 computer.

Concentrations of test chemicals in water were measured daily at each exposure level. Water was analyzed by gas chromatography (GC) in most cases with a few chemicals analyzed by UV spectroscopy and spectrofluorimetry.

GC analysis was performed on a Hewlett-Packard model 5730A gas chromatograph equipped with flame ionization and electron capture detectors. Columns for chemicals with water solubilities greater than 100 mg/L were generally packed with Tenax GC (Applied Science, Riviera Beach, FL). Less soluble compounds were first extracted into hexane or hexane/ether (75:25) and analyzed on an appropriate GC column utilizing either flame ionization or electron capture detectors. Column and injector temperatures were varied for each chemical to provide optimum analytical conditions.

Chemicals analyzed by UV spectroscopy were measured directly in water. The chemicals analyzed by spectrofluorometry were extracted with hexane prior to analysis.

Water solubilities of test chemicals were obtained from the literature or determined experimentally. Chemicals were added to water in 500-mL flasks and gently shaken in a 25 ± 1°C water bath until the concentration was constant. Samples of saturated water were centrifuged for 30 min at 11 000 × *g*, 31 000 × *g*, and 44 500 × *g* in separate polycarbonate or stainless steel centrifuge tubes. Water from each tube was removed for GC analysis. Samples centrifuged at more than 11 000 × *g* contained statistically ($P \leq 0.05$) identical concentrations as those centrifuged at 11 000 × *g*, and therefore, all water solubility values were treated as replicates.

TABLE 1. Acute toxicity (LC50) to fathead minnows, water solubility (C_s), *n*-octanol partition coefficient (P), and chemical activity at narcotic concentration (A_{nar}) for common alcohols (+, miscible with water; *, no fish mortality in saturated solution).

Chemical	96-h LC50 (mg/L)	log LC50 (m/L)	C_s (mg/L)	log C_s (m/L)	log P	LC50/C_s	A_{nar}[a]
Methanol	28 100	−0.06	+	+	−0.66	—	0.030
Ethanol	14 200 (15 400)[b]	−0.51	+	+	−0.16	—	0.027
2-Propanol	10 400 (9640)[b]	−0.76	+	+	0.14	—	0.025
1-Butanol	1 730	−1.63	74 700	0.03	0.88	0.023	0.022
1-Hexanol	97.5	−3.02	6 270	−1.21	2.03	0.016	
1-Octanol	13.5	−3.98	587	−2.35	3.03	0.023	0.020
1-Nonanol	5.7	−4.40	158	−3.00	3.53	0.036	
1-Decanol	2.4	−4.82	34	−3.67	4.03	0.070	0.055
1-Undecanol	1.04	−5.22	8.5	−4.31	4.53	0.122	
1-Dodecanol	1.01	−5.27	1.9	−4.99	5.00	0.532	
1-Tridecanol	*	*	0.33	−5.78	5.51	>1.0	

[a] Calculated from A_{nar} = LC50(mol/L) × activity coefficient presented in Amidon et al. (1974).

[b] Duplicate toxicity tests in parentheses.

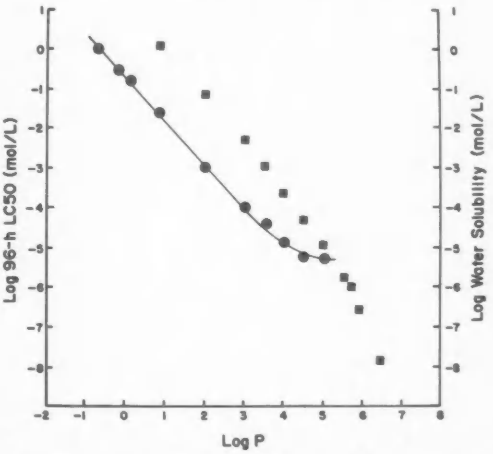

FIG. 1. Bilinear relationship between water solubility (squares) and 96-h LC50 concentrations (circles) for fathead minnows, *Pimephales promelas*, with *n*-octanol/water partition coefficient (log P) for some aliphatic alcohols.

The values for *n*-octanol/water partition coefficients were measured values taken from the monograph of Hansch and Leo (1979) and from a study by Veith et al. (1980). Those partition coefficients not previously measured were calculated using the fragment constant method of Hansch and Leo (1979) or estimated by reverse phase liquid chromatography as described by Veith et al. (1979).

Results and Discussion

A series of alkyl alcohols belonging to a class previously demonstrated to exhibit narcosis were tested to determine relationships among aquatic toxicity, water solubility, and log P of the chemicals. The log LC50 ranged from −0.06 mol/L for methanol to −5.27 mol/L for 1-dodecanol (Table 1). 1-Tridecanol produced no mortality at its solubility limit of 0.33 mg/L. The relationship is linear for homologs below 1-decanol and becomes nonlinear above decanol. The data are presented graphically in Fig. 1 for which the coordinates selected encompass essentially all of the common industrial chemicals. The square data points (Fig. 1) denote the water solubility and show that the linear relationship between solubility and log P presented by Hansch et al. (1968) can be extended to include some of the more insoluble alcohols such as dodecanol and tridecanol. The graph in Fig. 1 identifies "aquatic toxicity space" as that region below the water solubility. In the region above the water solubility, acute toxicity would be greater than water solubility and could not be measured under equilibrium conditions.

Acute toxicity and water solubility are covariant with respect to log P values less than 4.0 and have slopes of −1.04 and −1.09, respectively. The fact that toxicity is a relatively constant fraction of water solubility (chemical activity) suggests that the "Ferguson principle" (Ferguson 1939) of similar chemical activities producing similar effects in mammals is equally true for fish. Since narcosis in mammals is generally observed at chemical activities in the blood of 0.01−0.04 (Ferguson 1939; Mullins 1954), and in barnacle larvae at activities of 0.01−1.00 (Crisp et al. 1967), narcosis in fish would also be expected at 0.01−0.04 of the water solubility if fish were similar in sensitivity and an equilibrium existed between the water and fish blood. Table 1 shows that the ratio of LC50/C_s varies from 0.016 for hexanol to 0.036 for nonanol. The activity (LC50/C_s) of higher homologs increases until the LC50 is greater than water solubility. The activity of miscible chemicals cannot be estimated from water solubility; however, chemical activity at the LC50 can also be estimated by multiplying the LC50 by the activity coefficient of the chemicals. Although activity coefficient data in the literature are scarce, Amidon et al. (1974) presented coefficients for six of these alcohols. The activities necessary for narcosis in fish computed from the activity coefficients are presented in the last column of Table 1. Not only do these estimates show excellent agreement with the estimates from water solubility, but also, the mean activities of all lower homologs is approximately 0.025, which is similar to that needed for narcosis in mammals (Mullins 1954).

The increase in chemical activity observed for the higher homologs in this series may result from insufficient time to achieve a thermodynamic equilibrium between the water and lipid phases at water concentrations below 10^{-5} mol/L or from a decrease in the chemical activity of bulkier chemicals occupying larger molecular volumes (Mullins 1954). Experiments to test these hypotheses are in progress in this laboratory.

The data in Table 1 are summarized by the following bilinear equation (Kubinyi 1978):

$$\log \mathrm{LC50} = -1.09 \log P + 1.09 \log (0.000068P + 1) - 0.79$$

where $R^2 = 0.9986$. To test whether this relationship accurately models the toxicity of other industrial chemicals that are acutely toxic via narcosis, the 96-h LC50 of 50 other common chemicals from the industrial inventory were compiled (Table 2) and plotted on the alcohol model (Fig. 2). These data indicate general agreement between the narcosis equation using only log P as an independent variable and the observed LC50.

The least-squares equation for the entire data set is

$$\log \mathrm{LC50} = -0.94 \log P + 0.94 \log (0.000068P + 1) - 1.25.$$

This bilinear equation is the basic structure–toxicity relationship for computing the 96-h LC50 of chemicals that produce toxic effects by narcosis.

The ratio of observed/predicted LC50 values using this equation was 1.24 ± 0.94 ($n = 60$). The polynomial regression equation

$$\log \mathrm{LC50} = -1.10 \log P + 0.05 (\log P)^2 - 1.22$$

resulted in a similar level of predictability of toxicity, as the ratio of observed/predicted LC50 values using this equation was 1.29 ± 0.88 ($n = 60$). All of the predicted values were within one order of magnitude of the measured values, with a range for observed/predicted ratios from 0.12 for 3,3-dimethyl-2-butanone to 4.22 for 2-propanol.

TABLE 2. Acute toxicity of common industrial chemicals to fathead minnows (*, no fish mortality in saturated solution).

Chemical	96-h LC50 (mg/L)	log LC50 (mol/L)	log P
Alcohols			
2-Methyl-1-propanol	1430	−1.72	0.74[b]
2,2,2-Trichloroethanol	299	−2.70	1.38[c]
Cyclohexanol	704	−2.15	1.23[c]
2-Methyl-2,4-pentanediol	10700	−1.04	−0.70[b]
2-Phenoxyethanol	344	−2.60	1.16[c]
3-Furanmethanol (static)	508	−2.29	0.32[b]
Ketones			
Acetone	8120 (7280)[a]	−0.85	−0.24[c]
2-Butanone	3200	−1.35	0.28[c]
3-Pentanone	1540	−1.75	0.84[b]
2-Octanone	36	−3.66	2.46[b]
5-Nonanone	31	−3.66	3.00[b]
2-Decanone	5.7 (4.1)[a]	−4.44	3.54[b]
7-Tridecanone	*	*	5.16[b]
3-Methyl-2-butanone	864	−2.00	0.62[b]
5-Methyl-2-hexanone	159	−2.86	1.79[b]
4-Methyl-2-pentanone	505	−2.30	1.25[b]
6-Methyl-5-heptene-2-one	85.7	−3.17	2.13[b]
3,3-Dimethyl-2-butanone	87	−3.06	0.94[b]
Cyclohexanone	527 (732)[a]	−2.27	0.81[c]
Acetophenone	162	−2.87	1.66[c]
Benzophenone	15.3 (14.2)[a]	−4.08	3.38[c]
2′,3′,4′-Trichloroacetophenone	2.0	−5.05	3.73[b]
2′,4′-Dichloroacetophenone	11.7	−4.21	3.02[b]
2′-Hydroxy-4′-methoxyacetophenone	54.9	−3.48	2.14[b]
2′,3′,4′-Trimethoxyacetophenone	172	−3.09	1.12[d]
Ethers			
Triethylene glycol	69800	−0.33	−1.17[b]
2-(2-Ethoxyethoxy)ethanol	26400	−0.71	−0.08[b]
t-Butylmethyl ether	706	−2.10	1.30[b]
Diisopropyl ether	91.7	−3.05	1.56[b]

This structure–toxicity relationship is consistent with the "Ferguson Principle" of equipotency and the concept of a toxicity "cut-off" at the higher homologs (Ferguson 1939). The data show that log *P* is a much better molecular descriptor than the carbon number or molar volume used by Crisp et al. (1967), since all classes of chemicals fit a single relationship. The irregular relationships reported by Crisp et al. were simply due to the irregular relationship between carbon number and molar volume with log *P*.

The similarity between responses of fish and mammals to narcotic chemicals is likely the result of the nonspecific nature of the effect. Indeed, it may be expected that differences in the LC50 values among fish are small in comparison with the inherent errors in the QSAR. This is demonstrated in Fig. 3, which is a plot of the toxicity of industrial narcotic chemicals as measured by a 7-d LC50 with guppies (*Poecilia reticulata*) and reported by Konemann (1981), superimposed on the data in Fig. 2. The only apparent difference is that Konemann measured the toxicity of the more lipophilic chemicals such as pentachlorobenzene in the 7-d test, and the 4-d LC50 was not measurable for these chemicals. Konemann fitted the 7-d data to a linear relationship with log *P*. Because the water solubility decreases nonlinear with increasing log *P*, the Konemann data did not reflect the bilinear nature of the LC50–log *P* relationship for toxicity tests of shorter duration. Although the structure–toxicity relationship reported by Konemann (1981) is perfectly valid for estimating 7-d LC50 or 4-d LC50 for narcotics of lower log *P*, it may overestimate toxicity of chemicals for which the LC50 is very near the water solubility.

Predicting the mode-of-action of chemicals remains a difficult task, and the errors associated with selecting the wrong structure–toxicity mechanism may be greater than the standard error of the estimate associated with any individual QSAR. We recommend that the equation presented herein be used only for the classes listed in Table 2 or for chemicals for which there is evidence that they or analogous structures are narcotic. One other restriction is that, within the classes listed, structures that have a single functional group adjacent to the alcohol or carbonyl carbon may be more rapidly metabolized to a more toxic chemical resulting in computed toxicity much less than the actual toxicity. For example, 2-chloroethanol has a computed narcotic toxicity of more than 1000 mg/L but the measured LC50 is 37 mg/L. A likely metabolite is 2-chloroacetic acid, which is an enzyme inhibitor at levels below those that produce narcosis.

TABLE 2. *(Concluded).*

Chemical	96-h LC50 (mg/L)	log LC50 (mol/L)	log *P*
Di-*n*-butyl ether	32.5	−3.60	3.08[b]
2,6-Dimethoxytoluene	20.5	−3.87	2.67[b]
Diphenyl ether	4.0	−4.63	4.26[c]
Dipentyl ether	3.2	−4.70	4.16[b]
p-Nitrophenyl phenyl ether	2.7	−4.90	3.97[b]
Dioctyl ether	*	*	
Furan	61	−3.05	1.34[c]
Tetrahydrofuran	2 160	−1.52	0.46[b]
Alkyl halides			
1,2-Dichloroethane	118	−2.92	1.79[c]
1,1,2-Trichloroethane	81.7	−3.21	2.07[b]
1,1,2-Trichloroethylene	44.1	−3.47	2.42[e]
1,1,2,2-Tetrachloroethane	20.3	−3.92	2.39[e]
Tetrachloroethylene	13.5	−4.09	2.53[e]
Pentachloroethane	7.3	−4.44	3.64[b]
Hexachloroethane	1.5	−5.20	4.62[b]
Benzenes			
1,3-Dichlorobenzene	7.8	−4.28	3.38[c]
1,4-Dichlorobenzene	4.0	−4.56	3.37[e]
1,2,4-Trichlorobenzene	2.9	−4.80	4.28[b]
1,2,3,4-Tetrachlorobenzene	1.1	−5.29	4.99[b]
Pentachlorobenzene	*	*	5.71[b]
Hexachlorobenzene	*	*	6.42[b]
1-Fluoro-4-nitrobenzene	28.4	−3.70	2.02[b]
p-Dimethoxybenzene	117	−3.07	2.00[b]
3,4-Dichlorotoluene	2.91	−4.74	4.22[b]
α,α-2,6-Tetrachlorotoluene	0.97	−5.38	4.24[b]

[a] Duplicate toxicity tests in parentheses.
[b] Log *P* calculated by fragment constant method of Hansch and Leo (1979).
[c] Measured log *P* value reported in Hansch and Leo (1979).
[d] Log *P* estimated by HPLC as described by Veith et al. (1979).
[e] Measured log *P* value from Veith et al. (1980).

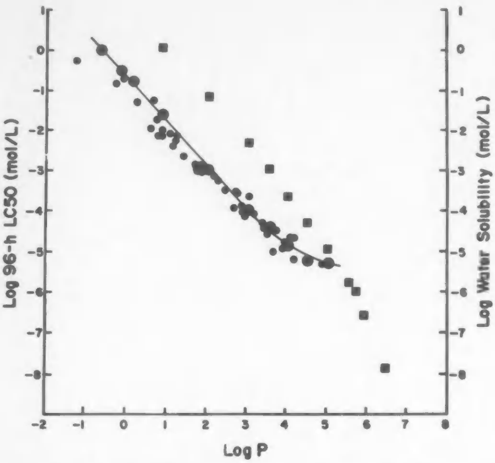

FIG. 2. Relationship between 96-h LC50 concentrations (small circles) for fathead minnows, *Pimephales promelas*, of some narcotic chemicals and log *P* superimposed on bilinear alcohol model.

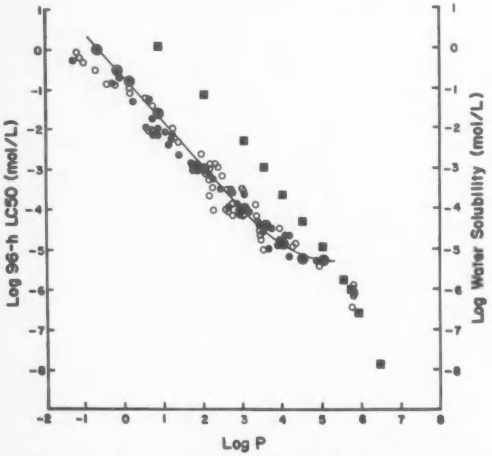

FIG. 3. Relationship between log 96-h LC50 concentration with fathead minnows, *Pimephales promelas*, and 7-d LC50 with guppies, *Poecilia reticulata*, for narcotic chemicals. Fathead minnow data (solid circles) from this study and guppy data (open circles) from Konemann (1981). Solid squares represent water solubility data.

In summary, the LC50 of industrial alcohols, ketones, ethers, alkyl halides, and substituted benzenes can be estimated by a single structure–toxicity relationship involving only the *n*-octanol/water partition coefficient. The chemical activities needed to produce lethality in fathead minnows are similar to activities needed to narcotize mammals. The structure–toxicity relationship for these narcotics in the fathead minnow is essentially the same as that reported for guppies and for barnacle larvae of *Elminius modestus*.

Acknowledgments

The work was supported by cooperative agreement CR809234-01 between EPA and the University of Wisconsin-Superior.

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Production Estimates for Scotian Shelf Copepods Based on Mass Specific *P/B* Ratios

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TREMBLAY, M. J., AND J. C. ROFF. 1983. Production estimates for Scotian Shelf copepods based on mass specific *P/B* ratios. Can. J. Fish. Aquat. Sci. 40: 749–753.

We estimated annual secondary production by copepod species on the Emerald Bank, Scotian Shelf, for the first time. We tentatively ranked copepod species in order of importance based on production to biomass (*P/B*) ratios calculated from adult body mass using an empirical relationship. The significance of assessing species in terms of production rather than population biomass is demonstrated. The mean estimate of total copepod production ($530\ kJ\cdot m^{-2}$) is in good agreement with previous estimates of secondary production on the Scotian Shelf.

TREMBLAY, M. J., AND J. C. ROFF. 1983. Production estimates for Scotian Shelf copepods based on mass specific *P/B* ratios. Can. J. Fish. Aquat. Sci. 40: 749–753.

Nous estimons pour la première fois la production secondaire annuelle par certaines espèces de copépodes sur le banc Émeraude du plateau Scotian. Ces espèces ont été provisoirement classées par ordre d'importance, sur la base des rapports production/biomasse (*P/B*) calculés à partir de la masse somatique des adultes à l'aide d'une relation empirique. Nous démontrons l'importance de l'évaluation des espèces en termes de production plutôt que de biomasse de population. L'estimation moyenne de la production totale de copépodes ($530\ kJ\cdot m^{-2}$) s'accorde bien avec des statistiques antérieures sur le plateau Scotian.

Received June 9, 1982
Accepted February 25, 1983

Reçu le 9 juin 1982
Accepté le 25 février 1983

PRODUCTION estimates for zooplankton on the Scotian Shelf are rare. Such estimates are difficult to obtain in open shelf areas characterized by continuously breeding populations. In other areas (both open seas and closed basins), production estimates have been made by various methods (for reviews, see Corkett and McLaren 1978; Greze 1978; Tranter 1976; Winberg 1971), among them, turnover rate methods (including the use of production to biomass (*P/B*) ratios) and physiological methods. All these methods have drawbacks, but the application of a *P/B* ratio is the least laborious. Given a *P/B* ratio for a particular group, all that is required is the mean biomass over the period of interest.

Mills and Fournier (1979) and Sameoto (1982) provided estimates of annual zooplankton production on the Scotian Shelf by applying a single *P/B* ratio to the whole zooplankton community (i.e. that sampled by an ~240-μm mesh net). Such estimates are useful, but more information is gained by applying species-specific *P/B* ratios. These result in an assessment of the importance of different species in energy flow, as well as a total production estimate by summation.

Banse and Mosher (1980) recently derived an empirical relationship between the annual *P/B* ratio and adult body mass. Herein we use the Banse and Mosher (1980) relationship to estimate annual *P/B* ratios for copepods of the Emerald Bank, Scotian Shelf. These *P/B* ratios are used in conjunction with estimates of mean annual biomass to give the first estimates of production by copepod species on the Scotian Shelf.

Copepods were selected for study because of their dominance in the zooplankton and because they are the most significant dietary component of larval fish in the area (L. J. Coates-Markle and J. C. Roff, unpublished observations). The study was carried out in cooperation with the Scotian Shelf Ichthyoplankton Program (SSIP) conducted by the Department of Fisheries and Oceans, which has as its goal a greater understanding of fisheries ecology on the Scotian Shelf.

Methods and Comment on Energy Equivalents

Thirty-one samples were obtained on or immediately surrounding Emerald Bank (Fig. 1) during eight SSIP cruises between November 1979 and October 1980 (see Tremblay and Roff 1983). Sampling involved towing 50-cm nets vertically through the water column at a speed of $30\ m\cdot min^{-1}$. Mesh sizes were 64 and 250 μm. Comparison of a series of samples obtained by the two nets (paired *t*-test) revealed that the 64-μm net undersampled the larger copepodites (>1.5 mm) but not significantly so. The 250-μm mesh nets significantly ($P < 0.01$) undersampled small species such as *Oithona similis* and *Microcalanus pusillus*, however, and correction factors were applied to the abundances of these two species when estimated from 250-μm mesh nets.

Samples were preserved in 5% formalin and seawater solution. The abundance and the individual dry weight were

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Printed in Canada (J6962)
Imprimé au Canada (J6962)

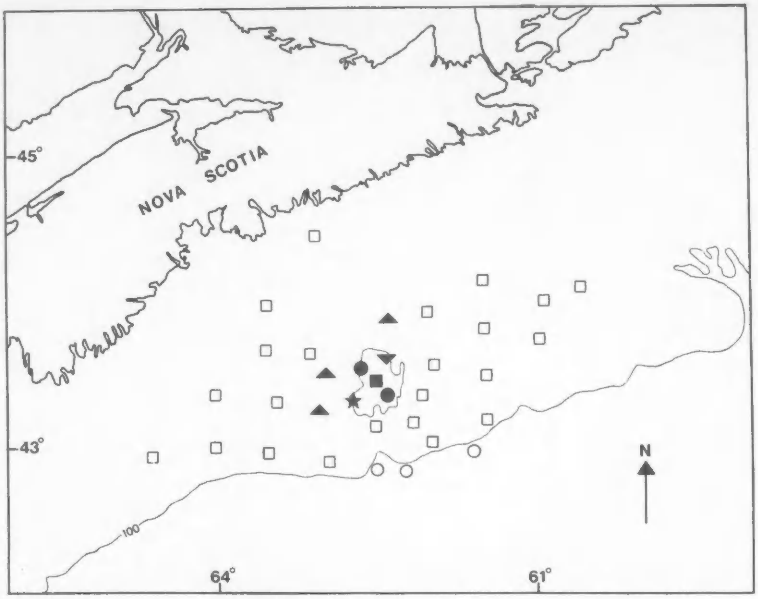

FIG. 1. Stations sampled for copepod production estimates on the Scotian Shelf (●, all cruises except November 1979; ■, all cruises except February 1980; ▼, all cruises except November 1979 and February 1980; ★, February 1980 only; ▲, November 1979 only) and additional stations sampled for total copepod biomass (□, shelf stations; ○, slope stations). Contour is 100 fathoms (~185 m).

estimated for the more numerous copepodites. We determined dry weights per copepodite to ± 0.5 μg using a Cahn electrobalance and corrected for preservation by assuming a 25% loss (Omori 1978). For the rarer copepodites (rarer species grouped as "others"), dry weights were obtained from the literature or estimated from length using the equations in Robertson (1968) and Evans (1977). The population biomass of each species was then computed for each sample and cruise means were determined. These cruise means were used to calculate mean annual biomass for each species. Interpolations were made to the middle of each month from November 1979 to October 1980 to avoid bias in the calculation.

To compare Emerald Bank stations with the surrounding Scotian Shelf, additional samples were obtained for estimates of total copepod biomass. Between February and September 1980 (see Tremblay and Roff 1983), 124 vertical hauls were taken (see Fig. 1 and Table 2) to a maximum sampling depth of 100 m at 5 Emerald Bank stations, 23 stations on the shelf, and 3 stations on the slope. Samples were preserved and treated as described above except that total copepod biomass only was calculated.

Banse and Mosher (1980) determined relationships between P/B ratios and adult body mass. Their equations differed depending on which data subset was used, e.g. they developed one equation for continuous breeders and another for discontinuous breeders. The equation used here was derived from the largest data set available ($n = 32$) and included invertebrates, weighing <1 mg to 1 g, that experience mean annual temperatures between 5 and 20°C. This equation (henceforth referred to as the BM equation) is as follows:

$$\log P/B = -0.16 - 0.34 (\log M)$$

where M is adult body mass in kilocalories (1 kcal = 4.1855 kJ). Ranges for the P/B estimates were obtained by taking 50 and 200% of the predicted value, as suggested by Banse and Mosher (1980).

Dry weight (grams) was translated into its energy equivalent; in marine copepods from shelf areas in the North Atlantic this is generally 21−29 kJ·g dry wt^{-1} (~5−7 kcal·g dry wt^{-1}) (L. J. Coates-Markle and J. C. Roff, unpublished observations; Comita et al. 1966; Laurence 1976). Energy values of aquatic organisms have been reviewed by Winberg (1971, chapter 2) who notes that "any values below 5 kcal·g^{-1} organic matter should be treated as suspect." The very low values, e.g. of Platt and Irwin (1968) and Mills and Fournier (1979), must be in error. For most crustacean zooplankton, which typically contain little carbohydrate, the lower limit of energy equivalents must be ~22 kJ·g ash-free dry wt^{-1} (~5.3 kcal), even allowing for 20% carbohydrate (17.6 kJ·g^{-1} or 4.2 kcal·g^{-1}), predominantly chitin. On a dry weight basis, allowing for 10% ash in copepods (Laurence 1976), this translates to a minimum estimate of about 20 kJ·g^{-1} (~4.9 kcal·g^{-1}). Values higher than 23.5 kJ·g ash-free dry wt^{-1} (~5.6 kcal, i.e. protein) will be due to increased lipid content (39.7 kJ·g^{-1} (~9.5 kcal·g^{-1}) (Comita et al. 1966). For example, a value of 34.7 kJ·g ash-free dry wt^{-1} (~8.3 kcal) for *Limnocalanus macrurus* from Lake Ontario in September (J. C. Roff, unpublished observations) represents a lipid content of approximately 70%.

The annual production for each species was estimated by determining the product of the P/B estimate and the mean annual biomass estimate. Other authors, notably Blueweiss et al. (1978) and Humphreys (1981), have also derived relationships between body size and production; the Banse and Mosher (1980) equation was used in preference because their data were based on a higher proportion of planktonic copepods.

Results and Discussion

Production estimates for the major copepod species and "others" are shown in Table 1. The overall ordering of species is tentative given the overlap in the ranges of the estimates. There is little doubt, however, that *Calanus finmarchicus* is the single most productive copepod. Comprising some 44% of the total, the annual production of *C. finmarchicus* is over three times that of the next species. *Oithona similis*, *Metridia lucens*, *Centropages typicus*, and *Pseudocalanus minutus* have similar levels of annual production (i.e. ranges broadly overlap). Together, the first five species make up almost 90% of the total annual production. *Paracalanus parvus*, comprising 4%, is transitional between the five dominants and the remaining copepods. *Calanus hyperboreus*, *Clausocalanus* sp., *Calanus glacialis*, and *Candacia armata* together comprise about 6% of the annual production by copepods on Emerald Bank. "Other" copepod species (Tremblay and Roff 1983) comprised approximately 0.8% of the total annual production (Table 1).

Populations of *C. finmarchicus*, *C. hyperboreus*, and *C. glacialis* all peak in May and June and show considerable seasonal variation in biomass (Table 1), whereas *O. similis*, *M. lucens*, and *P. minutus* are all seasonally much less variable. *Centropages typicus*, *P. parvus*, *Clausocalanus* sp., and *C. armata* all peak in the fall between August and November and also show considerable seasonal changes in biomass.

The total annual production by copepods on Emerald Bank amounts to approximately 593 kJ·100 m^{-3} (or 530 kJ·m^{-2}). The Emerald Bank had a significantly ($P \leq 0.05$) greater copepod biomass than the upper 100 m of the surrounding shelf or the upper slope during the periods sampled (Fig. 1; Table 2).

Some assessment of the validity of the P/B ratio estimates derived from the BM equation can be made by comparison with species-specific production estimates in the literature. The literature estimates for any one species tend to be wide ranging, however. The two estimates for *P. minutus* listed in Greze (1978), for example, were 58 for the Black Sea and 1.0–4.7 for the Norwegian Sea. Undoubtedly, some of this variability is due to location.

Banse and Mosher (1980) noted that animals living in polar environments tended to have lower P/B ratios (e.g. Rigler et al. 1974), while those living in tropical climates (annual temperatures greater than 25°C) tended to have higher P/B

TABLE 1. Production estimates for major species and for total copepods on the Emerald Bank. Estimates were derived using an equation from Banse and Mosher (1980). Caloric values are from Coates-Markle and Roff (unpublished observations).

Species	Adult body mass (kJ)	kJ (kcal)·g dry wt^{-1}	Mean (and annual range) of population biomass (kJ·100 m^{-3})	% of total biomass	Annual P/B ratio	Annual production (kJ·100 m^{-3})	% of total production
Calanus finmarchicus	0.0056	26.4 (6.3)	39.75 (3.71–120.29)	59.55	6.6 (3.3–13.2)	262.4 (131.2–524.8)	44.2
Oithona similis[a]	0.000047	22.2 (5.3)	2.47 (0.75–4.87)	3.70	31.7 (15.9–63.4)	78.3 (39.2–156.6)	13.2
Metridia lucens	0.0022	24.3 (5.8)	8.39 (4.82–12.11)	12.57	9.2 (4.6–18.3)	77.2 (38.6–154.4)	13.0
Centropages typicus	0.00069	22.6 (5.4)	4.64 (0.03–15.55)	6.95	13.0 (6.5–26.0)	60.3 (30.2–120.6)	10.2
Pseudocalanus minutus	0.00023	23.9 (5.7)	2.69 (0.98–3.97)	4.03	19.1 (9.5–38.2)	51.4 (25.7–102.8)	8.7
Paracalanus parvus	0.00015	23.0 (5.5)	1.06 (0.02–4.48)	1.59	22.0 (11.0–44.0)	23.3 (11.7–46.6)	3.9
Calanus hyperboreus	0.064	27.6 (6.6)[c]	4.85 (0–20.36)	7.26	3.0 (1.5–6.0)	14.5 (7.3–29.1)	2.5
Clausocalanus sp.	0.00017	23.4 (5.6)	0.64 (0.15–1.81)	0.95	21.1 (10.6–42.2)	13.5 (6.8–27.0)	2.3
Calanus glacialis	0.029	27.6 (6.6)[c]	1.67 (0–4.78)	2.50	3.9 (1.9–7.7)	6.5 (3.3–13.0)	1.1
"Others"	0.0013[b]	23.0 (5.5)	0.42 (0.12–1.06)	0.63	10.9 (5.5–21.8)	4.6 (2.3–9.2)	0.8
Candacia armata	0.0035	22.6 (5.4)	0.17 (0–0.71)	0.25	7.5 (3.7–14.9)	1.3 (0.6–2.6)	0.2
					Total[d] =	593.3 (296.7–1186.6) or 530.0 (265.0–1060.0) kJ·m^{-2}	

[a] Includes some early copepodites of *Oithona atlantica*.

[b] Weighted average based on species comprising 95% of "others" biomass (see Tremblay and Roff 1983 for species list).

[c] Estimated.

[d] Based on mean depth of 89.3 m on Emerald Bank.

TABLE 2. Results of Scheffe's means comparison procedure to test for differences in total copepod biomass $\log_{10}$ (g dry wt·100 m^{-3}) among cruise and station groups. A two-way analysis of variance showed that cruise and location were significant factors at $P < 0.0001$. Means followed by the same letter are not significantly different at $P \leq 0.05$.

	n	Mean
Cruise (season)		
May	20	6.22
June	22	3.50
August	21	1.52a
September	27	1.34ab
February	14	1.45bc
July–August	20	0.68c
Station group (location)		
Emerald Bank	26	3.25
Remainder of shelf	89	2.43
Slope	9	0.31

ratios. Variability in species-specific annual P/B estimates is also due to differences in age structure, food availability, and methodological differences. Banse and Mosher (1980) attempted to standardize the production estimates they used for the derivation of their equations by using only the results from studies that met certain criteria, e.g. mortality had to be accounted for, sampling had to be reasonably frequent, and extensive immigration and emigration had to be negligible.

For the dominant copepod, *C. finmarchicus*, lower and upper P/B estimates derived from the BM equation were 3.3 and 13.2, respectively, with a mean estimate of 6.6. Two P/B estimates for *C. finmarchicus* listed in Greze (1978) are 6.9 for the Barents Sea and 0.6–8.8 for the Norwegian Sea. Steele (1974) and Mills and Fournier (1979) made implicit estimates of 6–9 for the *C. finmarchicus* P/B. They applied this P/B to the whole zooplankton community by assuming that the main species, *C. finmarchicus*, has three generations per year and that because of mortality, the average zooplankton biomass is one-half to one-third the production of one generation. All of these estimates cover a range similar to that provided by the BM equation.

The P/B estimate derived from the BM equation for *P. minutus* was 19.1, with lower and upper limits of 9.5 and 38.2, respectively. The two estimates in Greze (1978) were noted earlier. Corkett and McLaren (1978) suggested that a daily P/B of 0.2 "might suffice in the North Sea for some purposes." Whether they implied an annual P/B of 73 (365 × 0.2) is unclear. The estimates derived from the BM equation for *O. similis* and *P. parvus* were 31.7 and 22.0, respectively. In the Black Sea, *O. similis* was found to have a P/B of 29.2 and *P. parvus* a P/B of 24.5 (Greze 1978). Given the above comparisons, we maintain that the P/B ranges derived for each species from the BM equation are reasonable.

Assessing species importance in terms of energy flow (production), rather than biomass, yields notably different results. In Table 1, *O. similis* has the second highest annual production. If the species are rated in terms of population biomass, however, *O. similis* is sixth behind *P. minutus*.

The importance of *C. finmarchicus* in annual production on Emerald Bank was expected. This species comprises approximately 80% of the copepod biomass during May and June, when copepod biomass is four to five times greater than the rest of the year (M. J. Tremblay and J. C. Roff, unpublished observations). The finding that *O. similis* was at least as important as *P. minutus*, *C. typicus*, and *M. lucens* was surprising. The small size of *O. similis* often either excludes it from zooplankton studies or causes it to be underestimated.

The first four species in Table 1, which comprise some 80% of the total annual production, have all been grouped as "intermediate" (Tremblay and Roff 1983), i.e. generally not correlated with temperature or salinity on the Scotian Shelf. The fifth species, *P. minutus*, was classified as "inshore" (Tremblay and Roff 1983) because it tended to have significant positive correlations with inshore conditions (lower temperature and salinity).

Studies in St. Margaret's Bay (Sameoto 1975) and the southwestern Scotian Shelf (Sameoto 1978) have indicated that *P. minutus* is one of the two most dominant species. That this was not the case on Emerald Bank may be due to two factors: the smaller mesh size of the nets used on Emerald Bank, which is more efficient at catching *O. similis*, and the fact that Emerald Bank is farther offshore than either St. Margaret's Bay or the southwestern shelf.

Species making up the remaining 11% of the annual copepod production were either "inshore" (definition above) or "offshore" (i.e. positively correlated with higher temperatures and salinities, Tremblay and Roff 1983). Inshore species were *C. glacialis* and *C. hyperboreus*; offshore species were *P. parvus*, *Clausocalanus* sp., most of the "others" category, and *C. armata*. Although these species contribute little on an annual basis, they can be important at certain times of the year. "Offshore" copepods alone can comprise up to 20% of the total copepod biomass in late summer and fall on the Emerald Bank (M. J. Tremblay and J. C. Roff, unpublished observations).

The Emerald Bank total copepod biomass was significantly greater than the rest of the shelf, but the average size composition of copepods was similar on the Emerald Bank and surrounding shelf areas (M. J. Tremblay and J. C. Roff, unpublished observations). Thus, P/B ratios should be similar in both areas, and the estimate of total copepod production for the Emerald Bank (530.0 kJ·m^{-2}·yr^{-1}) is probably higher than the average value for the shelf in the upper 100 m.

On the upper slope the average size of copepods would almost certainly be smaller than on the Emerald Bank; some approximate calculations indicated that the resultant P/B ratios would not be great enough to make the upper slope as productive as the Emerald Bank.

Mills and Fournier (1979) estimated annual secondary production on the Scotian Shelf as 89 kcal·m^{-2} (=373 kJ·m^{-2}) having assumed 1 g dry wt = 3 kcal. This is a low conversion factor considering the energy equivalents of organic matter and the very low ash contents of most zooplankters. Mills and Fournier's (1979) estimate translates to 745 kJ·m^{-2}·yr^{-1} (=178 kcal·m^{-2}·yr^{-1}) when assuming 1 g dry wt = 6 kcal (∼25 kJ), the approximate weighted mean caloric equivalent for Scotian Shelf copepods (L. J. Coates-Markle and J. C. Roff, unpublished observations).

Mills and Fournier (1979) suggested that their estimate of zooplankton production (which they termed herbivorous production) was "insufficient to provide a food base for the pelagic food chains." Our study, making use of information on individual species, and more realistic factors for converting grams dry weight to kilocalories, has resulted in an estimate of production by copepods some 50% higher than the original estimate (89 $\mathrm{kcal \cdot m^{-2}}$) of Mills and Fournier (1979) for total zooplankton.

Sameoto (1982) calculated the production of zooplankton on the Scotian Shelf as 1103 $\mathrm{kJ \cdot m^{-2} \cdot yr^{-1}}$ by assuming an average P/B ratio of 7. Since this was a mean of values for April and June only, the highest biomass months, this is probably an overestimate but still falls within our estimated range of values (Table 1). Also, our estimate is for copepods only and hence will be lower than for total zooplankton. Considering the uncertainties involved in making estimates of secondary production, it is remarkable that several estimates for the Scotian Shelf now agree within a factor of 2.

Empirical relationships for estimating P/B ratios, such as those in Banse and Mosher (1980), appear to be useful for assessing species importance and for estimating total production. Although the ranges for the production estimates are wide, the BM equation requires information that is relatively simple to obtain, and it can be applied to species whose life history patterns are presently unknown.

Acknowledgments

We thank Mr R. O'Boyle and Mr J. Reid, Marine Fish Division, B.I.O., Dartmouth; the Captains and crews of the *Lady Hammond*, *Brandel*, and *Letvlksm*; a contingent of University of Guelph students; and the staff of the Huntsman Marine Laboratory.This research was funded by DSS contract OSU78-00147 and a Natural Sciences and Engineering Research Council Canada grant to J. C. Roff.

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Multispecies larval fish associations: Accident or Adaptation?[1]

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FRANK, K. T., AND W. C. LEGGETT. 1983. Multispecies larval fish associations: accident or adaptation? Can. J. Fish. Aquat. Sci. 40: 754 – 762.

Capelin larvae (*Mallotus villosus*) time their period of larval drift to coincide with water masses containing abundant food resources and a reduced predator complex by responding to environmental cues associated with coastal water mass replacement. This persistent, coast-wide phenomenon led us to hypothesize that the initiation of larval drift in species of fishes other than capelin, having similar spawning modes, should be linked to the occurrence of these "safe site" water masses. The abundance of larvae of demersal spawning species, other than capelin, in the nearshore waters of our study site in eastern Newfoundland was highly variable during June – August in 1979 and 1980 and was unrelated to seasonal trends. The abundance of this sympatric noncapelin component of the ichthyoplankton was, however, positively related to both water temperature and larval capelin density, both of which are strongly influenced by coastal water mass replacement, and to the occurrence of conditions favorable to larval survival: "safe sites." The noncapelin larval assemblage was dominated by species having demersal eggs (90% of the total catch in two years) spawned in shallow water, in particular, winter flounder (*Pseudopleuronectes americanus*), radiated shanny (*Ulvaria subbifurcata*), and seasnails (*Liparis* spp.). Weekly size frequency distributions of these three species were dominated by newly hatched larvae. We conclude that the onset of larval drift in these species was also synchronized to the temporally variable occurrence of ecological safe sites for larvae. Three advantages accrue: food levels and potential growth rates would be greatly enhanced, predator density would be reduced, and predation rates would be reduced due to association with the dominant species (capelin) that saturate the predator field. Similar associations between a single dominant species and other much less abundant species appear to be common features of the ichthyoplankton of temperate/boreal regions.

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Les larves de capelan (*Mallotus villosus*) règlent leurs périodes de dérive larvaire de façon à coïncider avec les masses d'eau riches en nourriture et fréquentées par moins de prédateurs. Elles y arrivent en réagissant à des indices environnementaux associés avec le remplacement des masses d'eau côtières. Ce phénomène, persistant et répandu sur toute la côte, nous a conduit à l'hypothèse que le début de la dérive larvaire chez des espèces de poissons autres que le capelan, à modes reproducteurs identiques, serait lié à la présence de «sites sûrs» que représentent ces masses d'eau. L'abondance des larves d'espèces autres que le capelan, frayant sur le fond dans les eaux côtières que nous avons étudiées à l'est de Terre-Neuve a été très variable en juin – août 1979 et 1980, et ne montrait pas de relations avec les tendances saisonnières. Par contre, l'abondance ce cette composante sympatrique, autre que le capelan, de l'ichtyoplancton était en relation positive, à la fois avec la température de l'eau et la densité des larves de capelan, l'une et l'autre fortement influencées par le remplacement des masses d'eau côtières. Cette abondance est également influencée par la présence de conditions favorables à la survie des larves — «sites sûrs». Les communautés larvaires, autre que le capelan, étaient dominées par des espèces à oeufs démersaux (90 % des prises totales des deux années) libérés en eau peu profonde, en particulier plie rouge (*Pseudopleuronectes americanus*), ulvaire deux-lignes (*Ulvaria subbifurcata*) et limaces (*Liparis* spp.). La distribution hebdomadaire des fréquences de tailles de ces trois espèces étaient dominée par des larves nouvellement écloses. Nous concluons que le début de la dérive

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Printed in Canada (J7125)
Imprimé au Canada (J7125)

larvaire de ces espèces est également synchronisé avec la présence, variable dans le temps, de sites écologiques sûrs pour ces larves. Il en découle trois avantages : l'abondance de nourriture et les taux de croissance potentiels seraient beaucoup plus élevés; la densité des prédateurs serait moindre; et les taux de prédation seraient diminués, à cause de l'association avec l'espèce dominante (le capelan) qui sature le champ d'action des prédateurs. De semblables associations entre une espèce dominante unique et d'autres espèces beaucoup moins abondantes semblent être la caractéristique commune de l'ichtyoplancton des régions tempérées/boréales.

Received November 17, 1982
Accepted March 8, 1983

Reçu le 17 novembre 1982
Accepté le 8 mars 1983

LARVAL dispersal among most marine fishes plays a critical role in determining early larval survival. One consequence of effective dispersal is the positioning of larvae into habitats that, because of increased food densities in the edible size spectra and/or reduced predator densities, enhance survival. We have defined these areas as "safe sites" (Frank and Leggett 1982; after the terminology of Harper et al. 1961).

The occurrence of safe sites is typically spatially and temporally variable, and the occupation of spatially variable safe sites is often achieved through passive transport of eggs and larvae (see review in Frank and Leggett 1982; Parrish et al. 1981; Shelton and Hutchings 1982). In general, species with pelagic eggs tend to reproduce in proximity to historically consistent hydrographic phenomena such as gyres and fronts that increase food abundance (Harden Jones 1968; Loeb 1980; Owen 1981). An effective alternative strategy for occupying temporally variable safe sites is to reduce the magnitude of drift by producing demersal eggs or by vertical migrations between different current masses, the net effect of which is to retain larvae in estuarine or coastal environments (Pearcy and Richards 1962; Chenoweth 1972; Fortier and Leggett 1982). Such strategies would be favored when the probability of a suitable habitat reappearing locally is greater than that of finding a safe site at a distance.

Our early life history studies with beach spawning capelin (*Mallotus villosus*) indicate that this species copes with temporal variation in safe site occurrence by the latter strategy. Safe sites reappear intermittently and locally in the coastal embayments of eastern Newfoundland during the hatching period and result from wind-driven water mass replacement. Onshore winds result in the replacement of the cold, food-poor, macroinvertebrate predator-laden, upwelling waters that dominate the nearshore area during offshore winds with warmer, food-rich, predator-deficient surface waters (Frank and Leggett 1982). Although onshore wind events are infrequent and unpredictable, the timing of larval emergence and the onset of drift is strongly linked to it. The synchrony between the onset of larval drift in capelin and the occurrence of favorable feeding and predator conditions is achieved in the following way: onshore winds generate wave action capable of flushing larvae from the sediments and, simultaneously, elevated water temperatures enhance larval activity, which facilitates active escape. The interaction of these two variables produces a clearly defined synchronous transition to a planktonic existence and represents an adaptation to maximize feeding success and minimize mortality due to predation (Frank and Leggett 1982).

Have other coastal marine species timed their larval drift period to the occurrence of this safe site water mass? Association with this water mass would presumably convey to them advantages similar to those experienced by capelin. Furthermore, since the synchronous release of capelin larvae has the effect of swamping the predators (Frank and Leggett 1982), co-occurrence with large numbers of capelin larvae could further enhance the survival of relatively rare species if predation is a potentially important source of mortality in these species. In this paper, we examine the hypothesis that those species whose spawning mode is similar to that of capelin, and which co-occur with capelin (i.e. species having demersal eggs that are deposited in shallow water), will initiate their period of larval drift to coincide with the intrusion of surface water masses in the nearshore zone.

Methods

STUDY AREA

The study was conducted at Bryant's Cove, Conception Bay, Newfoundland (47°40′36″N, 53°22′24″W). A complete description of the study site is published in Frank and Leggett (1981a, 1982). Briefly, the cove is highly exposed and east-facing as are the majority of capelin spawning beaches on the east coast of Newfoundland and Labrador (Templeman 1948; Frank and Leggett 1982). The bottom contour is gently sloping from the beach to the mouth of the cove (distance = 750 m) where depths reach a maximum of 22 m. The bottom is generally composed of fine sand. Along the margins of the cove, sand is mixed among small and large boulders ranging in size from 0.5 to 3 m in diameter. This forms an extensive network of shelves and crevices. One major granite outcropping, extending to the surface at low tide, and rightfully termed the Sunker, forms a cone-shaped reef area (10–15 m in diameter) 500 m from the beach at a depth of 18 m.

HYDROGRAPHY

Temperature and salinity measurements for June–August from 1978 to 1980 are available in Frank and Leggett (1982). These data show that horizontal and vertical temperature differences in the cove are slight and nonsystematic during both onshore (north to east) and offshore winds. Thus, the nearshore waters are well mixed under both wind conditions.

FISH LARVAE

Plankton sampling was conducted at 1- to 3-d intervals in the cove during the June–August study period in 1979 and 1980. Samples were collected with a 0.5-m-diameter plankton net of 150-μm mesh fitted with a General Oceanics

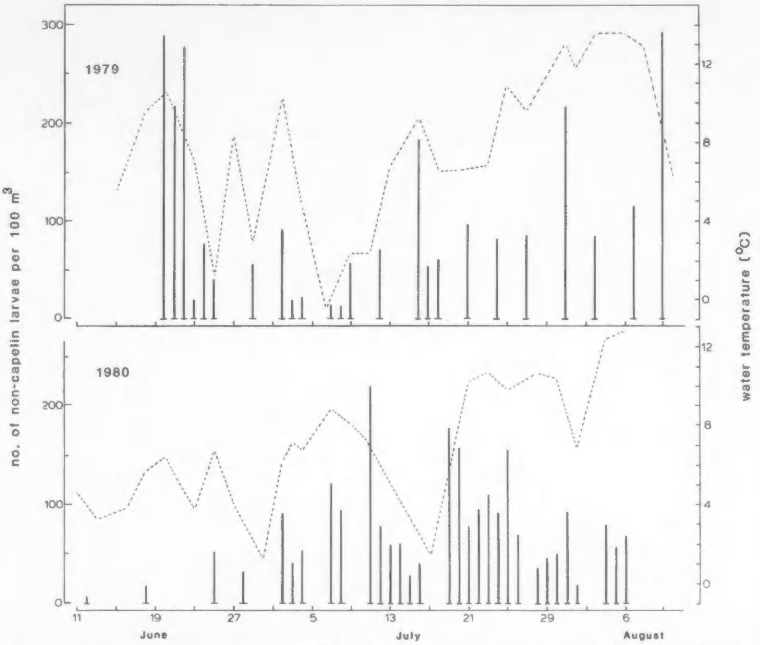

FIG. 1. Temporal changes in the combined abundance of noncapelin larvae in Bryant's Cove. Broken line shows water temperature in the 1−2 m depth stratum averaged across six stations in the cove.

flowmeter and towed horizontally (towing speeds averaged 1.2 ± 0.07 m/s) in the 1- to 2-m depth stratum. Six stations parallel to and at increasing distance from the beach starting at a distance 15 m from the beach, spaced equidistant to the mouth of the cove, and terminating approximately 730 m from the beach were sampled. Each station spanned the width of the cove. All samples were collected between 18:00 and 20:00 h and were preserved in 4% buffered formalin. All fish larvae were sorted from the samples and counted. Larvae other than capelin were identified and counted and length measurements (notochord or standard length) were determined to the nearest millimetre by the Larval Fish Identification Center, Huntsman Marine Laboratory, St. Andrews, New Brunswick. Average densities were calculated for each sampling date by weighting the combined catch against the volume filtered at each sampling station. Means were expressed as the number of larvae per 100 m^3.

Results

ABUNDANCE

The occurrence of larvae other than capelin alternated abruptly between episodes of high and low abundance during the June−August study period (Fig. 1). Systematic temporal changes in larval abundance were not evident. This is confirmed by the absence of any relationship between cumulative growing degree days (a measure of seasonal effect, Environment Canada monthly meteorological summaries) and absolute larval abundance for both 1979 and 1980 ($r^2 < 0.05$,

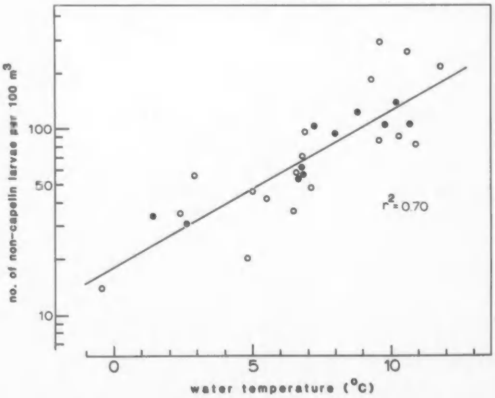

FIG. 2. The relationship between water temperature and density of noncapelin larvae collected in the nearshore zone. Sample size is the number of temperature measurements made in each year and is less than the number of sampling days for larvae. Daily abundances for certain periods therefore were averaged and related to the nearest temperature measurement. ○, 1979; ●, 1980.

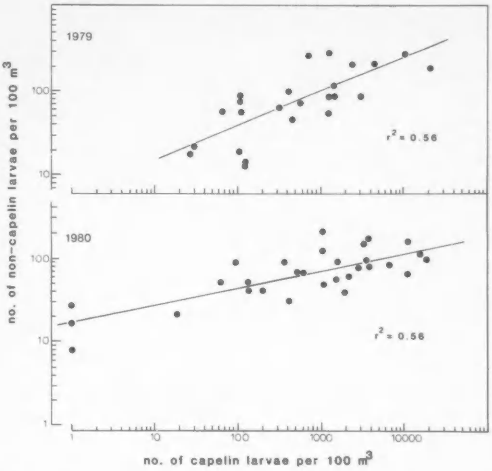

FIG. 3. Relationship between the density of non-capelin larvae and capelin larvae.

$P > 0.05$). There was, however, a significant relationship between larval abundance and water temperature, which was highly variable temporally. Most of the major peaks in abundance were associated with periods of rising water temperature (Fig. 1), indicative of onshore winds and the intrusion of a warm surface water mass. Regression analysis of the effect of water temperature (X, degrees Celsius) on the natural logarithm of larval abundance (Y, number per 100 m^3) revealed a significant positive relationship between these two variables ($N = 29$, $P < 0.001$) and yielded $r^2 = 0.70$ (Fig. 2). The equation to describe this relationship is

$$\ln Y = 2.898 + 0.1929X.$$

Strikingly few larvae were present in the nearshore zone during periods of upwelling (Fig. 1).

If indeed the relationship between the abundance of noncapelin larvae and water temperature is indicative of an association with safe site water masses, we would anticipate a positive relationship between the abundance of noncapelin larvae and capelin larvae, since capelin larvae are primarily associated with the warm surface water mass produced by onshore wind events (Frank and Leggett 1981a, 1982). A significant positive relationship was found between noncapelin larval density (Y, number per 100 m^3) and larval capelin density (X, number per 100 m^3) in both 1979 and 1980 (Fig. 3). The equation describing this relationship for 1979 is

$$\ln Y = 1.815 + 0.3958 \ln X,\ r^2 = 0.56,\ N = 24,\ P < 0.01$$

and the equation for 1980 is

$$\ln Y = 2.835 + 0.2035 \ln X,\ r^2 = 0.56,\ N = 30,\ P < 0.01.$$

The abundance of larvae other than capelin ranges between two and three orders of magnitude less than capelin larvae, when capelin densities exceed 1/m^3 (Fig. 3). The noncapelin larval component clearly makes a small contribution to the ichthyoplankton in this nearshore zone but is primarily associated with the occurrence of capelin larvae and the surface water mass.

There are at least two hypotheses to explain the observations in Fig. 2 and 3. Larvae other than capelin are simply broadcast at liberty into the environment and those occupying the surface waters experience relatively better survival than larvae in other water masses. This we will term the differential survival hypothesis. The second hypothesis is that the timing of emergence and initiation of drift is analogous to the strategy of capelin. If this hypothesis, which we call the adaptive emergence hypothesis, is true, we would anticipate the bulk of the species co-occurring with capelin larvae to originate from demersal eggs situated in the immediate shallow waters of the nearshore zone.

SPECIES COMPOSITION

The ichthyoplankton of Bryant's Cove consisted of larvae of 17 species in 1979 and 19 species in 1980, with 16 species occurring in both years (Appendix 1). In both years of the study the larvae were dominated by species with demersal eggs. Thirteen species with demersal eggs comprised 87 and 93% of the summer's total catch of noncapelin larvae in 1979 and 1980, respectively. The larvae of six species having pelagic eggs as well as representatives of the live-bearing *Sebastes* sp. were also present. Capelin larvae were the most abundant larvae in the cove, exceeding 95% of the total catch in each year. With capelin added to the total abundance tally, the representatives from pelagic eggs shrink to less than 0.1%.

Three noncapelin taxa with demersal eggs were most abundant in both years throughout the June–August study period: winter flounder (*Pseudopleuronectes americanus*), radiated shanny (*Ulvaria subbifurcata*), and seasnails (*Liparis* sp.). The combined catch of these three taxa exceeded 90% of the total on 18 of 24 and 24 of 30 sampling days during the June–August period in 1979 and 1980, respectively (Fig. 4). Exceptions to this general pattern occurred near the end of the study period when species derived from pelagic eggs (e.g. *Glyptocephalus cynoglossus* and *Limanda ferruginea*) were becoming dominant. This pattern agrees with other studies (Richards 1959; Herman 1963; Faber 1976), which indicate a seasonal progression of larval fish types from demersal to pelagic in coastal north Atlantic waters. Also, cottid and pholid larvae were prevalent initially in our 1980 collections. Although very few (<20/100 m^3) were collected (Fig. 1), this agrees with their known seasonal occurrence in other Newfoundland coastal regions (Ennis 1970). These data lend support to the adaptive emergence hypothesis because they uphold the prediction of a stationary egg type, a situation favored when suitable larval habitat reappears intermittently and locally rather than at a distance. The reported reproductive behavior for winter flounder, radiated shanny, and seasnails is also consistent with this hypothesis.

Winter flounder spawn primarily in May and early June in

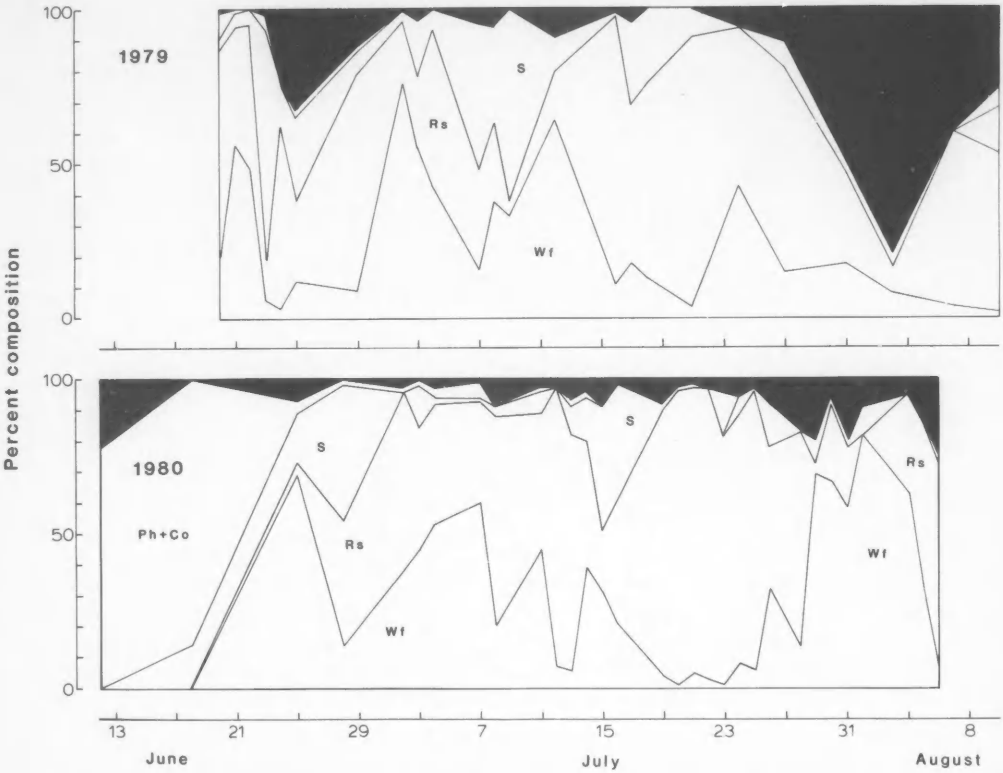

FIG. 4. Temporal changes in the species of fish larvae collected in Bryant's Cove. Unlabelled shaded areas represent all larvae from pelagic eggs. Unshaded areas show larvae from demersal eggs. Wf, winter flounder; Rs, radiated shanny; S, seasnails; Ph, *Pholis* sp.; Co, cottid larvae.

Conception Bay, Newfoundland, over a sandy bottom at depths less than 6 m (Kennedy and Steele 1971; Van Guelpen and Davis 1979). The small, spherical eggs (0.80 mm, modal diameter) sink to the bottom where they adhere in clusters. Hatching occurs after approximately 26 d at 4.5°C (Leim and Scott 1966). Radiated shanny spawn primarily in May and early June over sandy, rubble bottoms in Trinity and Conception Bay, Newfoundland (LeDrew and Green 1975). Fertilized eggs are 1.55 mm in diameter, demersal, and adhere to each other forming masses that rest on the bottom. Time to hatching ranges from 35 to 40 d at temperatures near 5.0°C. Faber (1976) reported late June and early July as spawning time for *Liparis* spp. in the Northumberland Strait region. Although temperatures were not specified, Detwyler (1963) estimated 22−30 d for hatching of *Liparis atlanticus*. *Liparis* spp. eggs are deposited in small clusters among rock crevices close to the tide mark and generally near shore (Bigelow and Schroeder 1953; Able and Musick 1976). We conclude that newly hatched larvae of these demersal spawning species are so located that they would experience the rapid temperature changes indicative of water mass replacement and the occurrence of safe sites and should be capable of responding to this stimulus in a manner similar to and synchronous with the larvae of capelin.

LARVAL SIZE DISTRIBUTIONS: WINTER FLOUNDER, RADIATED SHANNY, AND SEASNAILS

The rejection of either of the two hypotheses depends on the analysis of the size frequency distributions for each of the three species. The possibility remains that any one, or all, of the three dominant species reside in the surface water mass, which oscillates between the inshore and offshore regions and matches up with newly emergent capelin larvae when the prevailing winds are onshore. Temporal increases in the sizes of these larvae would confirm the existence of this situation and would tend to support the differential survival hypothesis.

Ninety-six percent of the total catch of winter flounder larvae during the June−August 1979 period was less than 4.0 mm and the size distribution of all of the weekly catches revealed a similar pattern (Fig. 5A). Although relatively fewer larvae were less than 4.0 mm in 1980 (77%) the pattern

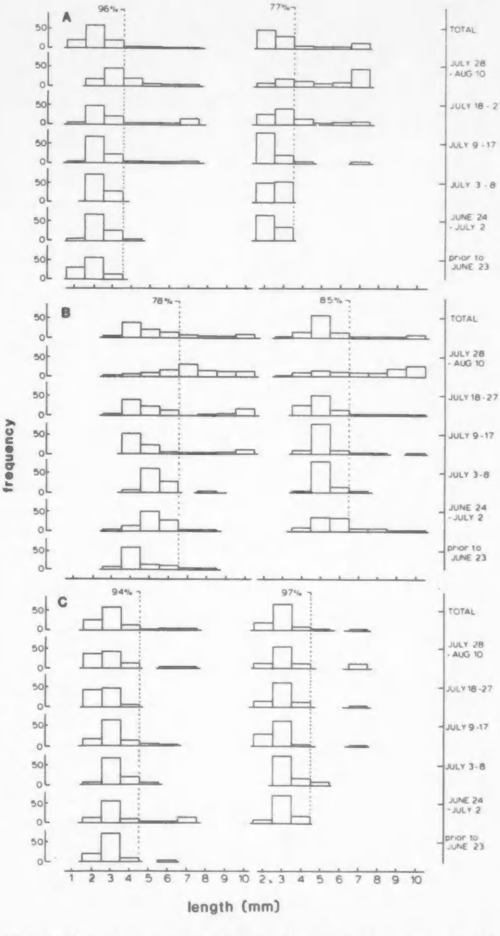

FIG. 5. Size frequency distributions for the dominant species of noncapelin larvae collected in 1979 (left-hand column) and 1980 (right-hand column). (a) Winter flounder; (b) radiated shanny, (c) seasnails. Broken vertical line denotes approximate size at hatching as reported from the literature. Larvae were grouped into 1.0-mm categories starting at 0.5 mm.

is equally striking throughout the month of July for the weekly size distributions. Yolk sac winter flounder larvae range between 3 and 3.5 mm (Bigelow and Schroeder 1953) and therefore the bulk of the winter flounder larvae occurring in the surface water mass are just beginning to initiate their period of larval drift.

Seventy-eight and 85% of all of the radiated shanny larvae were less than 7.0 mm in 1979 and 1980, respectively (Fig. 5B). These data also indicate that this species commences drift in association with the surface water mass, since LeDrew and Green (1975) reported yolk sac larvae of radiated shanny averaged 6.6 mm (range: 6.0−7.2 mm). Recently hatched individuals were evident in the weekly catches, especially during mid-June and July during 1979 and the entire study period for 1980. There was a tendency in the 1979 data, however, for the range of sizes represented in the collection to increase over time (Fig. 5B).

Seasnail larvae less than 5.0 mm dominated all of the weekly size distributions (Fig. 5C). This pattern was evident in both years, which resulted in 94 and 97% of the total catch being less than 5.0 mm for 1979 and 1980, respectively. Bigelow and Schroeder (1953) report that yolk sac larvae range in size between 3.3 and 4.5 mm. The implication of these size distributions is apparent.

All three taxa exhibited size frequency distributions dominated by individuals near to their reported size at hatching. This indicates that the larvae that associate with the surface water mass and co-occur with capelin larvae represent recently hatched larvae that enter the pelagic environment during coastal water mass replacement, which creates safe sites. These data are consistent with the adaptive emergence hypothesis and are inconsistent with the predictions of the differential survival hypothesis.

Discussion

We have previously shown that the initiation of larval drift in capelin is timed to the occurrence of wind-induced safe sites characterized by high concentrations of appropriate sized food and low macroinvertebrate predator density (Frank and Leggett 1982). The results of this study suggest that larvae of other demersal spawning species co-occurring with capelin exhibit identical behavior. The occurrence of these safe sites is temporally variable and unpredictable (Frank and Leggett 1981a, 1982). The strong association of larvae of these demersal spawners with safe sites, and/or co-occurrence with capelin, which dominate the ichthyoplankton community of these sites, implies that this timing is under strong selection and is therefore adaptive. The synchronous emergence strategy is an alternative to the match/mismatch hypothesis (Cushing 1975) because it embodies the role of short-term environmental uncertainty in shaping adaptive responses during the early life history rather than considering average conditions. Also, this alternative hypothesis encompasses a broader, more precise definition of what constitutes suitable larval habitat, the environmental correlates of such habitats, and the required species responses to achieve synchrony.

The trigger that synchronizes this association appears to be temperature (Fig. 2), which increases rapidly during onshore wind-driven, water mass replacement (Fig. 1). Capelin have been shown to respond to these increased water temperatures, which signal the arrival of safe sites, by increasing their swimming activity. This helps to synchronize emergence and insures association with conditions favorable to survival (Frank and Leggett 1982). It remains to be determined whether the larvae of the demersal spawning species studied here react similarly. In these species a sudden increase in water temperature could stimulate hatching, especially since the eggs are subjected to prolonged periods of low water temperatures resulting from persistent offshore wind conditions. Low temperatures also increase the variance in time to hatching among eggs of similar age (Frank and Leggett 1981b). This fact, combined with an extended spawning period of 2–4 wk (Kennedy and Steele 1971; LeDrew and Green 1975; Faber 1976), produces a protracted period of larval release apparently linked to the occurrence of onshore wind events and ecological safe sites. Turbulence created by onshore wind-driven surface waves may also contribute to synchrony by producing an uplifting dispersive effect during water mass replacement. Cottid larvae occupying nearshore areas (depth 6 m) have been shown to be dispersed by wave action in this manner (Ennis 1970). It is probable that the larvae of winter flounder, radiated shanny, and seasnails are similarly affected.

It is important to note that the situation we describe for Bryant's Cove is not merely a local phenomenon. During the summer of 1982 we recorded nearshore water temperatures from six capelin spawning locations on the east coast of Newfoundland between LaScie (49°58′N, 55°36′W) and Witless Bay (47°16′N, 52°49′W). Changes in nearshore water temperatures, indicative of water mass replacement, were in synchrony, within 1–2 d over this region. This reflects the scale (100–1000 km) of the atmospheric pressure systems that drive coastal upwelling leading to the creation of safe sites. This supports our earlier hypothesis that the occurrence of conditions favorable to survival may be synchronous over large geographic areas (Frank and Leggett 1982).

We propose three selective advantages of synchronous initiation of larval drift in these demersal spawning species other than capelin. First, by synchronizing their emergence to the occurrence of wind-driven safe sites the larvae enter into a water mass rich in food in the edible size spectrum. In the case of capelin, the increase in edible plankton resulting from water mass replacement may increase growth rate up to fivefold (Frank and Leggett 1982). Similar acceleration of growth rates is likely to accrue to noncapelin larvae that occupy these safe sites. A second advantage is reduced predation pressure. Surface water masses, which replace the deeper upwelling water masses during onshore wind events, are characterized by a macroinvertebrate predator complex reduced 3- to 20-fold over that contained in the upwelling deepwater mass (Frank and Leggett 1982). A third advantage, also related to predator avoidance, may accrue to species that synchronize the initiation of larval drift to specific environmental conditions. We have previously shown (Frank and Leggett 1982) that the synchronous release of capelin in high densities has a swamping effect on certain macroinvertebrate predators (e.g. Ctenophores), thereby reducing daily mortality rates by up to fivefold. By synchronizing the onset of larval drift with that of capelin, which are 100- to 1000-fold greater in abundance (Fig. 3), larvae of less abundant species such as winter flounder, radiated shanny, and seasnails may benefit from this predator swamping phenomenon. Examples of increasing immunity from predation with declining population size, when overall abundance is high, has been shown to accrue to such ecologically unified groups as mixed (heterotypic) schools of bait fishes (Nursall and Pinsent 1969; Ehrlich and Ehrlich 1973; Ogden and Ehrlich 1977; Keenleyside 1979) and to several terrestrial animal species (Sweeney and Vannote 1982; Karban 1982).

This co-occurrence of species of lesser abundance with a dominant species appears to be a widespread phenomenon. Seasonal ichthyoplankton surveys conducted off the Atlantic coast of North America reveal numerous such complexes in which the dominant species varies with latitude (Richards 1959; Herman 1963; Pearcy and Richards 1962; Chenoweth 1972; Faber 1976). Such associations are evident both in the spring (cold water) ichthyoplankton peaks dominated by larvae of demersal spawners and in the summer (warm water) peaks dominated by larvae of pelagic spawners. These large-scale survey data do not permit a detailed evaluation of the short-term character of such associations or the identification of factors producing them. The broad pattern revealed does, however, support the concept that, for species having similar egg types and depth distributions, synchrony in the timing of life history events is adaptive and is to be expected. It is possible that the protection from predation afforded by co-occurrence with a dominant species significantly enhances survival, thereby contributing to species richness and community stability. Birch (1981) has recently demonstrated that such is the case in many marine benthic communities.

Acknowledgments

Principal financial support for this research was provided by Fisheries and Oceans Canada, Science Subvention Program. Significant supplementary funding was provided by the Faculty of Graduate Studies and Research, McGill University, and by operating and strategic grants from the Natural Sciences and Engineering Research Council of Canada. Logistic and other assistance was provided by the Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada, St. John's, Newfoundland. We are especially indebted to Dr J. E. Carscadden of Fisheries and Oceans Canada, Mr Mike Dolan, and Kathleen W. Frank for their insights and assistance in various phases of our work. Susan Bocti contributed to the preparation of the manuscript. Finally, we acknowledge our indebtedness to the residents of Bryant's Cove, Newfoundland, for their hospitality and frequent assistance.

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Appendix

Species of larvae collected in Bryant's Cove during June–August in 1979 and 1980

	1979	1980	Egg type (d = demersal; p = pelagic)
Pleuronectidae			
Pseudopleuronectes americanus	x	x	d
Limanda ferruginea	x	x	p
Hippoglossoides platessoides	x	x	p
Glyptocephalus cynoglossus	x	x	p
Stichaeidae			
Ulvaria subbifurcata	x	x	d
Stichaeus punctatus	x	x	d
Osmeridae			
Mallotus villosus	x	x	d
Clupeidae			
Clupea harengus harengus	x	x	d
Scorpaenidae			
Sebastes sp.	x	x	live bearer
Cyclopteridae			
Liparis sp.	x	x	d
L. atlanticus	x	x	d
L. inquilinus	—	x	d
Pholidae			
Pholis gunnellus	x	x	d
Cottidae			
Gymnocanthus tricuspis	—	x	d
Myoxocephalus aenaeus	x	x	d
Myoxocephalus scorpius	x	x	d

Myoxocephalus octodecemspinosus	—	x	d
Ammodytidae			
Ammodytes sp.	x	—	d
Gadidae			
Gadus morhua	x	x	p
Enchelyopus cimbrius	x	x	p
Labridae			
Tautogolabrus adspersus	x	x	p

Use of the *Pisces IV* Submersible for Zooplankton Studies in Coastal Waters of British Columbia

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MACKIE, G. O., AND C. E. MILLS. 1983. Use of the *Pisces IV* submersible for zooplankton studies in coastal waters of British Columbia. Can. J. Fish. Aquat. Sci. 40: 763–776.

This study evaluates the usefulness of a small submersible for observations of the plankton. A method for calculating plankton densities from estimates of mean interanimal distances is described. Estimates made by this method were compared with estimates based on net sampling and were found to be in fair general agreement with them. Fragile gelatinous forms were better counted from the submersible, small organisms by netting. Some delicate species, known to be abundant from submersible observations, were never recognized in net samples. Submersible observations also gave important insights into vertical distribution of the plankton. Several species were found to exist within unexpectedly narrow and sharply defined layers, often at densities greatly surpassing density estimates based on net samples. In Saanich Inlet, B.C., plankton distribution was studied in relation to the seasonal formation and dispersion of the oxygen-deficient basin water. Other data deal with behavior, color change, bioluminescence, and vertical migration of planktonic organisms. We conclude that submersible observations are potentially valuable in plankton research, and we make recommendations regarding instrumentation and observer training as an aid in planning future dives.

MACKIE, G. O., AND C. E. MILLS. 1983. Use of the *Pisces IV* submersible for zooplankton studies in coastal waters of British Columbia. Can. J. Fish. Aquat. Sci. 40: 763–776.

Le but de la présente étude est d'évaluer l'utilité d'un submersible de petite taille pour observer le plancton. On y décrit une méthode de calcul des densités du plancton à partir d'estimations de distances moyennes entre animaux. Nous comparons les estimations obtenues par cette méthode avec celles d'échantillonnages au filet et trouvons assez bonne concordance entre les deux. Il est plus facile de dénombrer les formes gélatineuses fragiles depuis le submersible, alors que les petits organismes le sont plus facilement par échantillonnage au filet. C'est ainsi que certaines espèces délicates, que des observations par submersible prouvent abondantes, ne sont jamais identifiées dans les échantillons des filets. En outre, les observations par submersible donnent une très bonne idée de la distribution verticale du plancton. On constate que plusieurs espèces vivent dans des couches inopinément étroites et nettement définies, souvent à des densités dépassant de beaucoup les estimations fondées sur les échantillons prélevés au filet. Dans l'inlet Saanich (C.-B.), nous avons étudié la distribution du plancton en relation avec sa formation saisonnière et la dispersion de l'eau du bassin, pauvre en oxygène. Les autres données portent sur le comportement, le changement de coloration, la bioluminescence et la migration verticale des organismes planctoniques. Nous concluons que les observations par submersible offrent beaucoup de possibilités comme outils de recherche sur le plancton, et faisons certaines recommandations relatives aux appareils et à la formation d'observateurs, dans le but d'aider à la planification des plongées futures.

Received May 11, 1982
Accepted March 1, 1983

Reçu le 11 mai 1982
Accepté le 1 mars 1983

DEEP submersible research vessels have been used for marine biological investigations since about 1953, mainly for work on the benthos, as in the explorations by *Alvin* in the Galápagos rifts (Corliss et al. 1979). Observations on planktonic organisms have occasionally been reported, but typically, these deal with rare and interesting animals seen in the course of dives made for other purposes. The discovery and capture of rhodaliid siphonophores ("dandelions") by *Alvin* (Corliss et al. 1979; Clark 1979) is one example, others being the observation and collection of a new scyphozoan medusa by *Deepstar* (Russell 1967), of a new ctenophore by *Alvin* (Madin and Harbison 1978), of pelagic holothurians by *Turtle* and *Sea Cliff* (Barnes et al. 1976), and of giant larvacean houses by *Deepstar* (Barham 1979).

Printed in Canada (J6930)
Imprimé au Canada (J6930)

Observations on unusual aggregations of siphonophores (Rogers et al. 1978), on feeding mechanisms (Youngbluth 1982), and on the composition of the deep-scattering layers (e.g. Barham 1963, 1966; Milliman and Manheim 1968) represent contributions to plankton ecology in a more general sense. However, to find submersibles being used for systematic reporting of the overall distribution of a wide range of zooplankton species in the water column, we must go back to the pioneering studies of Bernard (1955) and Trégouboff (1956, 1958, 1961, 1962), both of whom used the Piccard submersible *F.N.R.S. III*, predecessor to the *Trieste*. A complete bibliography of the work of *F.N.R.S. III* up to 1957 is given by Fage (1958). A contribution by Franqueville (1970) follows in the same tradition.

The Canadian *Pisces* submersibles (MacDonald 1975) have been used quite extensively for studies of the marine benthos, the most recent example being Tunnicliffe (1981). The few reports covering the plankton are brief and are restricted to generalities (e.g. Herlinveaux 1970; Levings and McDaniel 1973).

The present series of studies using *Pisces IV* had as its objectives (1) the further pursuit of Herlinveaux's (1970) goal of assessing the potential of *Pisces* submersibles for biological oceanography, (2) the development of more quantitative approaches to visual reporting of plankton composition, and (3) the use of these methods to examine certain specific aspects of plankton biology in British Columbia coastal waters.

Dive Sites

Locations of the dives are shown in Fig. 1.

Saanich Inlet — A total of 25 dives by one or both authors in company with other observers were carried out between August 1980 and April 1981. Saanich Inlet, on the southeast side of Vancouver Island, is 24 km long and 7.2 km wide at its widest part and about 200 m deep in the central region for most of its length. There is a sill at the mouth, where the depth is 75 m (Herlinveaux 1962; Deuser 1975). The deep water inside the sill is isolated for much of the year and undergoes progressive oxygen depletion, along with reduction of nitrates, nitrites, and sulphate and production of hydrogen sulphide. Upwelling off the open coast leads indirectly to an annual influx of dense water, which may cross the sill and enter Saanich Inlet, displacing between 18 and 46% of the water in the basin (Anderson and Devol 1973). Oxygen data for 1980–1981 are given by Tunnicliffe (1981). This flushing is usually restricted to the months of August and September. The new water descends to the bottom of the basin or to an equilibrium point near the bottom. After flushing, oxygen depletion commences. Conditions of low oxygen concentration, plus the presence of hydrogen sulphide in this zone, make it generally inhospitable to animal life, though the benthic community has been shown to exhibit a remarkable tolerance to periodic low-oxygen conditions (Tunnicliffe 1981).

Stuart Channel — Lying directly north of Saanich Inlet, but lacking a sill and open at both ends, Stuart Channel was chosen as a dive site for comparison with Saanich Inlet. It is well oxygenated to the bottom. Dives were conducted between March 30 and April 13, 1981.

Jervis Inlet — Data were collected in dives on May 21, 1980, September 29, 1980, and February 22–23, 1981. Jervis Inlet is characterized by low runoff. There is no glacial silt in the water. Seasonal cycles in temperature, salinity, and oxygen are minor below the top 10 m (Pickard and Stanton 1980).

Knight Inlet — The data included here were obtained by Dr V. J. Tunnicliffe during dives concerned mainly with observation of epilithic faunal assemblages. Knight Inlet carries a high glacial sediment load. Despite the greater amount of freshwater entering Knight than Jervis, there is little difference in temperature, salinity, and oxygen content between the two inlets below 10 m (Pickard and Stanton 1980). A sill at Hoeya Head rises to 63 m from the surface. The plankton observed above and below the sill appeared similar, and the data have been combined for the present account.

Howe Sound — The data reported here were collected by Dr V. J. Tunnicliffe during dives in March 1981. Howe Sound has a 40-m sill in Montagu Channel, which partially isolated the 275-m-deep inner basin from the rest of the inlet. The outer water is similar to water from the Strait of Georgia. Basin water undergoes severe oxygen depletion at certain times (Bell 1973, 1974), but at the time of the present dives, mixing was evidently in progress or had recently occurred, as the water contained in excess of 1 $mL \cdot L^{-1}$ throughout the water column. The distribution of the plankton appeared similar in the inner basin and outer channel, and the sightings have been combined in the present report.

Methods

DESCRIPTION OF *PISCES IV* SUBMERSIBLE

Constructed in 1972 by International Hydrodynamics of North Vancouver, *Pisces IV* is owned by the Department of Fisheries and Oceans (Canada) and is based at the Institute of Ocean Sciences, Sidney, B.C.

The cabin of the submersible measures approximately 2 m in diameter, which allows spaces for a pilot and two observers. It can operate to depths of 2000 m. Depending on the power drain, dives of 4–8 h are regularly undertaken. The life support system can operate for 216 person-hours. Observations are made through three ports (15 cm inside diameter, 36 cm outside diameter). Two 1000-W lights are mounted outside the observer's ports, and two 500-W lights are mounted above the pilot's port. Adjustment of the angle of these lights is essential for accurate observation of the plankton.

A hydraulic manipulator arm is mounted ahead of the pilot port, along with other instrumentation. Water samples can be taken through a valve in the hull during dives.

The *Pisces IV* has a variable buoyancy system that includes both a hard ballast system, in which small buoyancy adjustments are made by pumping water in and out of ballast chambers, and a soft ballast system, operating on compressed air. This versatile system allows for rapid, small adjustments in the vessel's buoyancy and fine control of the rate of ascent and descent, the need for which has been noted by users of some other submersibles, where such adjustments were not so convenient.

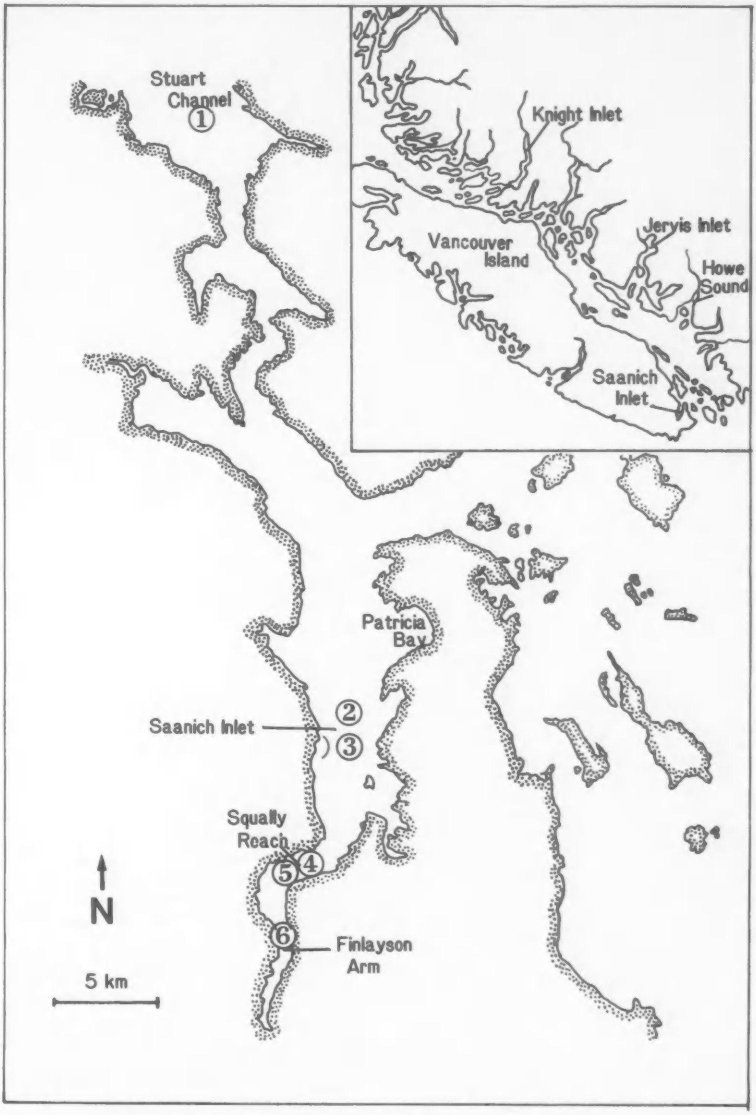
Stuart Channel
①
Knight Inlet
Jervis Inlet
Vancouver Island
Howe Sound
Saanich Inlet
Patricia Bay
②
Saanich Inlet
③
Squally Reach
⑤
④
N
⑥
Finlayson Arm
5 km

FIG. 1. Sites on the coast of British Columbia where dives were made. Station 1 (209-m depth) lies equidistant between Crofton and Vesuvius Bay; station 2 (200−210 m) lies off Yarrow Point; station 3 (210 m) lies off Tozier Rock; station 4 (220 m) lies off McCurdy Point; station 5 (215 m) lies off Elbow Point; station 6 (200 m) is in the middle of Finlayson Arm.

COLLECTION, IDENTIFICATION, AND COUNTING OF PLANKTON

On two occasions, plankton samples were taken at the dive site using a 75-cm-diameter closing net with 333-μm mesh. Vertical hauls were made over known depth ranges, and the density and composition of the plankton was determined for comparison with estimates made by direct observation from *Pisces*. Water samples taken during dives were analyzed for oxygen content by the Winckler procedure.

Visual observations made from the submersible during

TABLE 1. Density of organisms estimated from observed average center-to-center distances between organisms. Number per cubic metre is calculated from the formula: number of organisms/volume (m^3) = 1.41/distance (m^3) and assumes that closest packing of spheres with an organism at the center of each sphere is an appropriate model for the distribution of these organisms.

Observed average center-to-center distance between organisms	Calculated No. · m^{-3}
1 cm	1 410 000
2 cm	176 250
3 cm	52 222
4 cm	22 031
5 cm	11 280
10 cm	1 410
15 cm	418
20 cm	176
30 cm	52
40 cm	22
50 cm	11
1 m	1.41
1.5 m	0.42
2 m	0.18

TABLE 2. Principal species referred to in the text.

- Hydromedusae
  - *Aequorea victoria*
  - *Phialidium gregarium*
  - *Euphysa japonica*
  - *Proboscidactyla flavicirrata*
  - *Aegina citrea*
  - *Aglantha digitale*
- Siphonophora
  - *Muggiaea atlantica*
  - *Dimophyes arctica*
  - *Nanomia cara*
- Ctenophora
  - *Pleurobrachia bachei*
  - *Pleurobrachia* species *B* (determination pending)
  - *Bolinopsis ?infundibulum* (determination pending)
  - *Beroe abyssicola*
- Pteropoda
  - *Clione limacina*
- Polychaeta
  - *Tomopteris pacifica*
  - *Tomopteris septentrionalis*
- Copepoda
  - *Aetidius divergens*
  - *Calanus* spp.
  - *Euchaeta elongata*
  - *Metridia pacificus*
- Amphipoda
  - *Cyphocaris challengeri*
  - *Parathemisto pacifica*
  - *Orchomenella affinis* (= *O. obtusa*)
- Euphausiacea
  - *Euphausia pacifica*
- Decapoda
  - *Munida quadrispina*
- Chaetognatha
  - *Sagitta elegans*
- Appendicularia
  - *Oikopleura dioca*
  - *Oikopleura labradoriensis*

dives were recorded verbally on tape recorders. Photography of plankton through the ports was attempted using the external strobe light, but the photographs lacked sufficient clarity to be useful for purposes of quantification and identification.

Sources for identification of species included Fulton (1968), Shih et al. (1971), and Kozloff (1974). Identification of medusae was aided by reference to Arai and Brinkmann-Voss (1980). For pelagic polychaetes, reference was made to Tebble (1962) and Ushakov (1974), and for decapod larvae, Hart (1971) was referred to.

In preliminary trials, three observers were assigned to report independently on plankton distribution and density at the same site on successive days. The observers were familiar with the local plankton but only one of them had previous submersible experience. From these trials, methods were selected for counting the plankton and displaying the results graphically. In most dives, a tubular plastic frame delineating a space of known cubic dimensions was mounted in front of each observer port, and the observers counted or estimated the numbers of the organisms in this space.

In the data for Saanich Inlet, densities are shown graphically by spots. The density of spots gives a rough indication of the density of the organism at a particular depth in relation to its density, and that of other species, at other depths and times, but these data are not to be interpreted in terms of absolute values. Where densities could not be systematically estimated (data from Jervis, Howe, and Knight inlets) only the range is given.

In later dives, densities were calculated from estimates of the average center-to-center distance between organisms. Numbers of organisms per unit volume can be calculated on a model of closest packing of equal spheres, assuming each animal to be at the center of a sphere. In either hexagonal or face-centered closest packing of spheres, the spheres occupy 74% of the total volume (Van Vlack 1964). Thus, the number of spheres (N) times the volume of a sphere equals 74% of the total volume:

$$(N)(\tfrac{4}{3}\pi r^3) = (0.74)(\text{volume}).$$

Therefore, for N organisms:

$$N/\text{volume} = 0.74/\tfrac{4}{3}\pi r^3.$$

Thus

$$N/\text{volume} = 1.41/d^3$$

will yield densities of planktonic animals based on d, the observed average distance between animals (in centimetres or metres, corresponding to volume in litres or cubic metres, respectively).*

A set of distances and equivalent densities covering the range encountered in the *Pisces* work is provided in Table 1.

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*NOTE ADDED IN PROOF: Hamner and Carleton (1979) provide a formula relating average nearest neighbor distance to density based on the assumption of isahedronic packing.

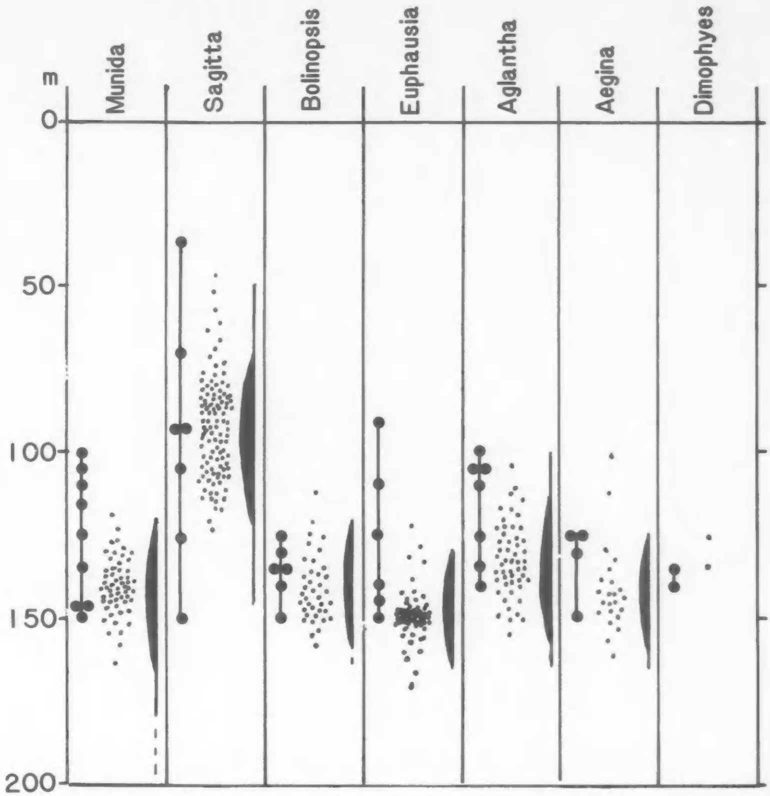

FIG. 2. Agreement among observers. Distributions of selected species were plotted independently by three naïve observers. The dives were conducted during the early afternoon during the period between August 18 and August 20, 1980, at station 3, Saanich Inlet.

During dives, a graduated scale was placed outside each observer port as an aid in judging distances.

The reliability of this method of estimating densities was tested in experiments conducted in laboratory tanks using known concentrations of suspended objects (plankton, fish eggs, etc.). The most convenient test objects proved to be "model medusae": fish liver oil capsules fixed in formalin to render the gelatin insoluble, washed, and suspended in tap water to which ethanol was added to make the capsules neutrally buoyant. Capsules were added arbitrarily to produce different densities, which were then estimated by four observers using the interanimal center-to-center distance formula. Afterwards, the actual numbers of capsules were determined and their densities per cubic metre calculated directly. The water in the tank was kept in gentle irregular motion during the counts.

Results

The principal species observed during the study are listed in Table 2.

OBSERVER AGREEMENT IN *PISCES* OBSERVATIONS

Results from trials conducted at the beginning of the study are shown in Fig. 2. Observer 1 recorded plankton sightings as single spots, chiefly with a view to establishing range, indicating regions of high abundance by a double spot. Observer 2 indicated abundance by spot density, as described under Methods. Observer 3 showed abundance in another manner. It will be apparent that there was fairly good agreement between the sightings. The differences may, in part, represent true differences in plankton distribution on the three occasions when the dives were made.

A possible source of confusion in some dives is that, in descending or moving forwards through the water column, the submersible carries a body of water with it containing trapped organisms. In addition to such passive displacements, some organisms such as euphausiids, which are strong swimmers and are positively phototactic, cluster around the lights and follow the submersible downward during slow descents. Observer 1 (Fig. 2) disregarded sightings of *Munida*, *Euphausia*, etc. below about 150 m, believing that

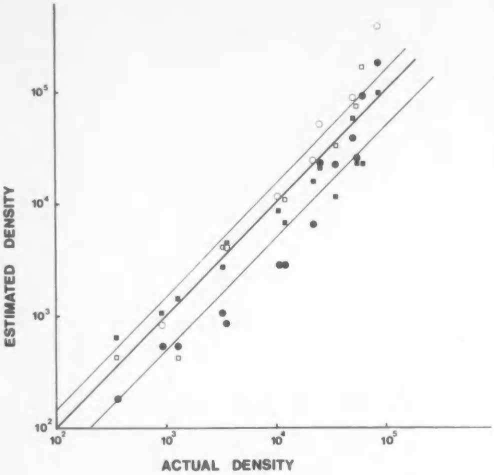

FIG. 3. Density estimates by four observers in model medusa experiment. Density units are numbers of animals per cubic metre. Correct estimates would fall along the center line (50% error limits are shown on either side). Solid circles and squares are estimates by the two authors.

their presence there was due to movement of the submersible or its lights. This was later shown to occur on a number of occasions. In later dives, when we were attempting to establish precise limits to the zonation, we made a point of coming up from below as well as going down into areas we were interested in and of keeping the lights off during ascents and descents, except when counts were to be made. It was sometimes found advantageous to maintain slow forward movement with the thrusters while ascending or descending. The submersible thus enters new water all the time, and forms that are attracted or repelled by light do not have sufficient time to move toward or away from the advancing lights. Trimming the submersible with the bow up minimizes the forward trapping effect.

LABORATORY TESTS ON RELIABILITY OF FIELD DENSITY ESTIMATES

Actual densities and estimates by four observers are compared in Fig. 3. The agreement of observers' estimates with actual densities, and the agreement between observers, was evaluated by correlation using the Pearson product–moment correlation coefficient, which ranges from 0 (no relationship) to 1.0 (perfect relationship). Over the set of 14 test densities our estimates correlated 0.86 and 0.87 with actual values and 0.88 with each other. While a few estimates differed from actual values by over 100%, the overall mean percentage error was 53%. The greatest inaccuracies were noted at the highest actual density values ($>60\,000 \cdot m^{-3}$). Considering only the estimates of densities below this value, mean percentage error was 37%.

COMPARISON OF NET HAULS AND *PISCES* OBSERVATIONS

On November 4, 1980, and March 30, 1981, plankton tows were carried out in Saanich Inlet for comparison with submersible counts made concurrently. Fair agreement between the two data sets was found on both occasions. Table 3 lists the data for hydromedusae, siphonophores, and ctenophores from the November 4 counts.

As also noted by Franqueville (1970), net sampling is clearly more effective in the case of small transparent objects like eudoxids, which could not be accurately counted from the submersible but were scored as "few" or "common" (the same would apply to copepods and crustacean microplankton). However, observations from the *Pisces* revealed a considerably higher density of ctenophores than did the net counts, and one common species (*Pleurobrachia* species B) was not recognizable at all in the net samples, presumably because of disintegration under the stress of capture. The submersible showed higher numbers for *Aegina* probably for the same reason. The November data were obtained at a time when the plankton was dispersed over a much greater vertical range than during most of the year, when anoxic water limits distribution in the lower levels. In March, submersible observations showed several species to be present in high concentrations in narrow bands over the anoxic zone. The data for *Aglantha* are shown in Fig. 4 compared with data from plankton hauls. Similarly, in August 1981, *Bolinopsis* reached densities of $20 \cdot m^{-1}$ within a narrow layer at 145–150 m. Because the net integrates the densities from all the levels it traverses, it fails to reveal the sharp discontinuities and very high local concentrations seen by observers in the submersible. With prior knowledge, or good luck, net samples might be taken at just the right depths to reveal the stratification precisely, but the fact remains that in none of the present series of net hauls and in none of the previous reports is there any adequate indication of the precision of the layering or the high densities achieved within these narrow bands. The advantages of the submersible are quite clear in this regard.

SUBMERSIBLE OBSERVATIONS FROM SAANICH INLET, STUART CHANNEL AND MAINLAND FJORDS

The observations from Saanich Inlet and Stuart Channel have been taken from the more comprehensive study of Mills (1982) and are intended to serve as examples illustrating the usefulness of submersible observations.

Figure 5 shows plankton distribution as observed from the submersible for three periods, during two of which the water below a certain depth was seriously deficient in oxygen. On the other occasion, mixing had occurred. The plankton was limited to water containing more than 0.2 mL $O_2 \cdot L^{-1}$ with the exception of an amphipod, *Orchomenella affinis*, already shown by Hoos (1970) to be tolerant of anoxia.

During periods when the water column was adequately oxygenated to the bottom, most species were also distributed all the way to the bottom, but some, notably *Euphausia pacifica*, continued to show a concentration at an intermediate depth.

It was very obvious from the submersible observations that

TABLE 3. Comparison of plankton densities estimated from net samples (two at each depth) and counted by eye from the submersible in Saanich Inlet, Nov. 4, 1980.

Species	Plankton tows		Visual counts	
	Depth (m)	No.·m^{-3}	Depth (m)	No.·m^{-3}
Hydromedusae				
Aegina citrea	75–130	0.04, 0	100–185	0.2–0.5
	130–180	0.09, 0		
Aglantha digitale	0–25	0, 0.09	80–185	2–10
	25–50	0, 0.09		
	75–130	2.60, 2.15		
	130–180	2.27, 1.86		
Phalidium gregarium	0–25	0.09, 0.09	Surface only	<0.5
Proboscidactyla flavicirrata	0–25	0, 0.27	0–75	<0.2
	25–50	0.18, 0.09		
	50–75	0.27, 0		
Siphonophora				
Dimophyes arctica (polygastric)	50–75	0.36, 0.18	60–100	<0.2
	75–130	0.12, 0.17		
Dimophyes arctica (eudoxids)	50–75	0.09, 0.18	140–170	Few
	75–130	0.29, 0.29		
	130–180	0.18, 0.28		
Muggiaea atlantica (polygastric)	0–25	1.09, 0.91	15–34	0.5
	25–50	2.00, 3.45	35–47	5.0
	50–75	2.73, 5.82	48–70	0.5
	130–180	0.05, 0		
Muggiaea atlantica (eudoxids)	0–25	5.54, 5.46	30–60	Common
	25–50	9.00, 14.63		
	50–75	2.73, 5.82		
	130–180	0.05, 0		
Nanomia cara	50–75	0.09, 0	165–180	Only two seen
Ctenophora				
Bolinopsis ?infundibulum	50–75	0, 0.09	20–185	0.2–0.5
	130–180	0, 0.05		
Pleurobrachia bachei	0–25	0.09, 0	0–75	<0.2
	25–50	0.09, 0		
Pleurobrachia species B	None recognizable		100–154	0.2–0.5
			155–165	5
			166–185	0.2–0.5

the zone just above the anoxic layer, whether this fell at 160 or 100 m, contained a high concentration of plankton.

In general, the plankton distribution at the two ends of Saanich Inlet was closely similar during the period in question (Fig. 6), but marked differences were observed between Saanich Inlet and Stuart Channel.

Oikopleura dioica was undergoing an intense bloom at the time in question, reaching densities of 10 000·m^{-3}, as recorded on another occasion by Seki (1973). Its distribution was similar at all three sites. *Aglantha* and *Euphausia* were located in narrow layers around the 100-m mark. The concentrations of these forms were much higher in Saanich Inlet than in Stuart Channel. *Oikopleura labradoriensis*, *Sagitta*, *Bolinopsis*, and the two species of *Pleurobrachia* all showed a greater downward range in Stuart Channel than in Saanich Inlet, which must be partly attributed to the absence of an oxygen-deficient zone in Stuart Channel, supporting similar findings by Hoos (1970). The greater upward dispersion of several species in Stuart Channel suggests that certain weakly swimming forms are less able to maintain a "preferred" depth in a water mass subject to tidal mixing.

Night dives were successfully conducted on two occasions, making it possible to compare night and daytime distributions within a single 24-h period (Fig. 7).

The results confirm that *Euphausia* and *Sagitta* migrate toward the surface at night, as previously shown by net sampling (e.g. Arai and Fulton 1973) and as also shown for euphausiids and chaetognaths in the Mediterranean (Franqueville 1970) and in Norwegian fjords (Hopkins and Gulliksen 1977). It was also noted that *Munida* is a migrator. *Aglantha* migrated on one occasion, but not on the other. This is interesting in view of the existence of conflicting reports (reviewed by Mackie 1980) from various parts of the world as to whether *Aglantha* migrates or not, and in which direction.

Oikopleura labradoriensis may be a migrator, but the redistribution was not as obvious as in the other cases mentioned. There are indications in our records that both species of *Pleurobrachia* may be migrators, but numbers were too small for this to be established with certainty. Some of the amphipods and copepods definitely migrate, but because of difficulties with identification, these results are omitted.

Diel changes in the depth of components of the sound

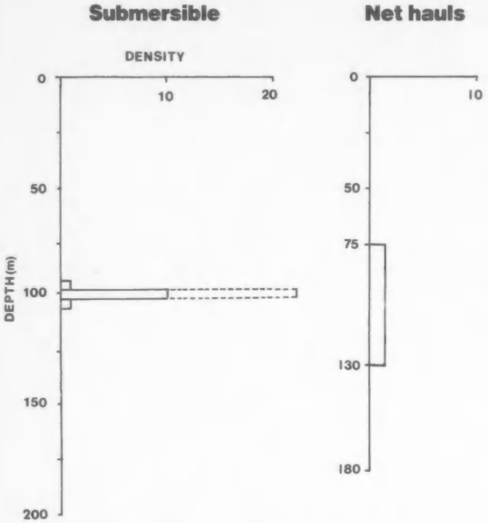

FIG. 4. Distribution of the medusa *Aglantha digitale* at station 2, Saanich Inlet, March 30, 1981. Comparison of visual density estimates from the submersible and density estimates obtained by plankton tows. Density units are numbers of animals per cubic metre; depth units are metres.

scattering layers in Saanich Inlet have generally been interpreted in terms of the movement of fishes (e.g. Herlinveaux 1962; Bary 1967), but the presence of invertebrate plankton, particularly euphausiids, in very high concentrations just above the oxycline at certain times suggests that they alone may be sufficient to produce the scattering even with relatively low-frequency sounders (see calculations by Hersey and Backus 1962). There seems to be little doubt that euphausiids produce a midwater scattering layer as detected by high-frequency echo sounding in Saanich Inlet (Bary and Pieper 1970) and in other areas (Pieper 1979).

The most striking feature observed during the *Pisces* dives in Saanich Inlet was the extraordinarily high concentrations of euphausiids just over the oxycline (see Fig. 5). The highest previous estimates for Saanich Inlet are $280 \cdot m^{-3}$ (Bary 1966) and $250 \cdot m^{-3}$ (Boden and Kampa 1965). We have found local concentrations of euphausiids in bands of 2–3 m thickness reaching densities as high as $10\,000 \cdot m^{-3}$, equivalent to a center-to-center spacing of approximately 5 cm. Net sampling can easily miss such concentrations as in the case of *Aglantha* (Fig. 4). Care was taken in these observations to distinguish natural aggregations from concentrations induced by the submersible's lights. Table 4 gives data from various dives. Usually, there was a single high-density band, but on one occasion, two bands were seen. Euphausiid densities of this order of magnitude have previously been reported only in surface swarms (Terazaki 1980).

Some indication of the precision of the banding can be given by quoting from detailed notes made after a daytime dive at station 2 on March 30 (Mills 1982):

> "Above 99 m, only isolated individuals were seen; at 99 m, density was approximately $10\ m^{-3}$, and an observer could look down from the submersible into a cloud of *Euphausia* that began rather abruptly at 100 m, where, within a meter, the density increased from about $100\ m^{-3}$ to $10,000\ m^{-3}$. Density began declining below 102 or 103 m, so that only 100–$400\ m^{-3}$ were present by 108 m. At 112 m, the density was less than $10\ m^{-3}$, and by 115 m only a few scattered *Euphausia* were left."

Opportunities to dive in Jervis Inlet, one of the mainland fjords, were too limited to allow more than a bare cataloguing of the more obvious planktonic species. Similarly, the sightings for Howe Sound and Knight Inlet are of a preliminary nature, but they serve to show that, in many respects, the spring plankton in the three inlets is similar. Exceptions exist; for instance, the absence of *Munida* larvae in Knight Inlet. Adult *Munida* are rare here, possibly because of the high sediment load. Figure 8 shows the ranges of selected species. Squid and fish are omitted, as the focus here is on the plankton community in the narrower sense.

The distributions of the species observed in Jervis Inlet may be considered under four general categories:

Group 1 (epipelagic) — Examples are *Muggiaea*, rarely seen below 100 m, and *Phialidium*. In the September 1980 dive, *Muggiaea* showed an upper distribution limit at 35 m, probably the bottom of the halocline. However, *Proboscidactyla* and *Aequorea* were present above the halocline, and *Pleurobrachia bachei* was distributed on both sides of it down to 40 m.

Group 2 (upper mesopelagic "narrow band" forms) — *Aglantha*, *Munida*, and *Euphausia* fall within this group, showing distributions similar to those of their conspecifics in Saanich Inlet.

Group 3 (mesopelagic "broad band" forms) — These include all except one of the remaining forms shown in Fig. 8. Of these, *Nanomia* and *Euphysa* showed a deeper distribution as adults than as young. *Aegina* young, by contrast, occurred deeper than the adults. A second species of *Tomopteris*, presumed to be *T. septentrionalis*, and *Dimophyes artica* also belong in group 3, along with two unidentified ctenophores, a colorless beroid, and a large elongate cydippid. Finally, a *Lensia*-like diphyid and a small agalmid siphonophore, about half the size of a mature *Nanomia*, colorless and deep living, were quite conspicuous in the lower group 3 range.

Group 4 (bathypelagic) — The only representative of the Jervis plankton that can be assigned to this class is *Beroe abyssicola*, which appears to be confined to the aphotic zone, and shows the dark red coloration typical of such forms.

GENERAL OBSERVATIONS

The opportunity to observe midwater animals feeding, swimming, and interacting in their natural habitat is one of the great attractions of submersible operations. The presence of a motionless submersible is unlikely to affect the behavior of

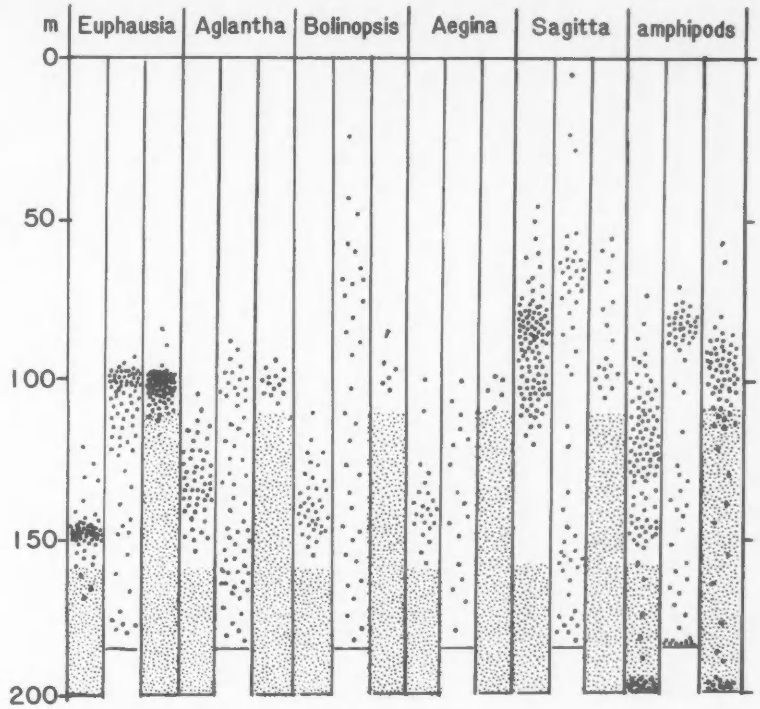

FIG. 5. Distribution of selected species in relation to the oxycline, Saanich Inlet, station 2. The three columns under each species give data for August 20–22, November 5–7, 1980, and March 30–April 3, 1981, respectively. Stippling shows oxygen-deficient zone (<0.2 mL $O_2 \cdot L^{-1}$).

most planktonic species, except insofar as the lights may produce responses. Mackie (1974) questioned some of Barham's observations of siphonophore behavior on these grounds, but we can now confirm the validity of Barham's report on the behavior of *Nanomia*, having observed the same activities under conditions where the lights could not have been responsible. Barham (1970) suggested that some marine organisms are sensitive to electric signals produced by submersibles, but this possibility has yet to be systematically explored.

Many members of the zooplankton are normally in a state of restless activity, and this applies not only to crustacean microplankton but to some larger, gelatinous forms as well. Eudoxids and polygastric stages of *Muggiaea* and medusae such as *Aglantha* have been seen performing the rhythmic feeding cycles associated with food collection seen in the laboratory. *Aglantha* also perform the rapid escape type of swimming (Donaldson et al. 1980) in conditions that suggest that escape from potentially damaging contact with other organisms is frequently called for and effectively executed. *Aegina* are seen swimming with the tentacles clustered "teepee" fashion above the bell, very much as illustrated for *Aeginopsis* by Pérès (1959). *Bolinopsis* usually hang vertically, with their oral lobes down, beating their combs enough to maintain this position. Occasionally, the ctenophore contracts its lobes suddenly and swims off rapidly to one side. *Pleurobrachia* species *B* can cruise slowly with tentacles outstretched or more rapidly (approximately 2 $cm \cdot s^{-1}$) with them contracted. *Tomopteris* appear to swim continuously. *Sagitta* make spasmodic darting movements. *Munida* larvae hang with chelae held vertically over their heads. *Oikopleura* beat their tails in a slow rhythm while in their houses but swim in rapid bursts when outside.

According to Franqueville (1970), the zooplankton is in perpetual movement, ascending and descending, except during a short period around the middle of the day. This observation was not verified in the present study, but alternating ascents and descents were observed in *Aglantha*.

Munida larvae are white during the day at their midwater depth, but on rising to the surface at night, they are red, doubtlessly due to chromatophore expansion. The photoprotective advantage attributed to such changes in copepods (Hairston 1980) can hardly apply here. It is of interest that another pelagic galatheid, *Pleuroncodes*, also performs vertical migrations (Boyd 1967) and shows diel changes in coloration (C. M. Boyd, Dalhousie University, personal communication).

Previous submersible users in British Columbia waters

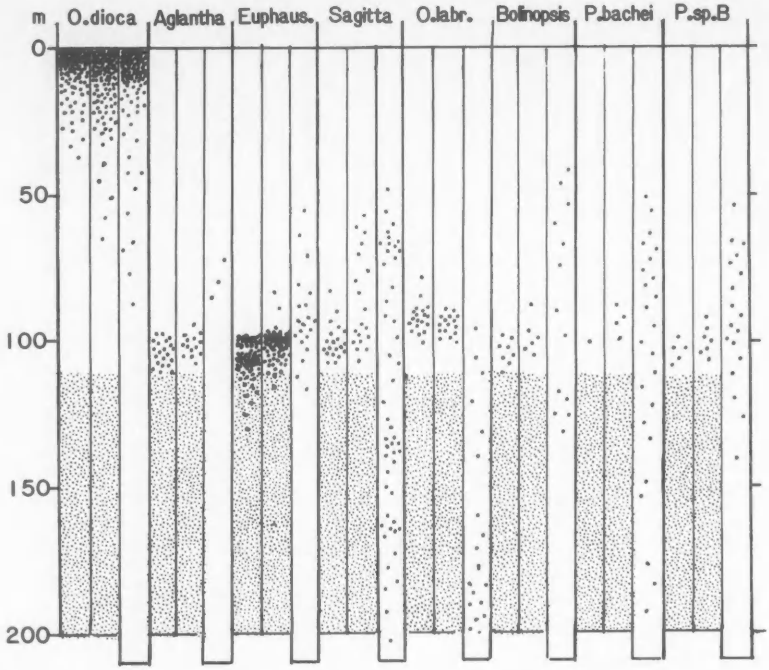

FIG. 6. Distribution of selected species in Saanich Inlet compared with Stuart Channel, March 30–April 3, 1981. Under each species, the three columns represent, respectively, stations 4, 5, and 6, stations 2 and 3, and station 1. Stippling shows dysaerobic zone of Saanich Inlet.

have commented on the abundance of "mucus" in the water. Herlinveaux (1970) suggested that some such objects were decomposing ctenophores. Levings and McDaniel (1973) regarded them as clusters of dead phytoplankton. The material in question occurs in the form of sheets up to 10 cm wide, filaments, and medusiform "parachutes" and presumably corresponds to the "marine snow" of some authors (e.g. Silver et al. 1978; Caron et al. 1982). The aggregates were most frequently observed just above the oxycline in Saanich Inlet, where they achieved densities as high as $500 \cdot m^{-3}$. They were present in lower concentrations in the anoxic layer. Aggregates seen in the upper water were usually small and may correspond to the microbial clumps reported by Seki (1971). Zonation of such aggregates has been described in waters off Toulon (Pérès and Piccard 1956) and St. Tropez (Franqueville 1970).

Very fine particles were conspicuous in the basin water of Saanich Inlet, forming milky clouds toward the top of the oxygen-depleted water. Elemental sulphur is reported to occur in the anoxic water (R. H. Herlinveaux, Institute of Ocean Sciences, Sidney, B.C., personal communication), but manganese oxide and associated bacteria may also be present at the oxycline (Emerson et al. 1979).

Finally, crustacean exoskeletons and abandoned *Oikopleura* houses were frequently seen from the submersible. Considering the abundance of euphausiids in the inlet and the fact that they molt throughout life, there must be a substantial fallout of exoskeletal debris entering the anoxic layer.

Light emission from *Oikopleura* was very striking near the surface at night, when these forms were abundant. The empty houses as well as inhabited ones luminesce, as noted by Galt (1978). Flashes due to hydromedusae such as *Phialidium gregarium* and to euphausiids were of common occurrence but were not systematically studied.

During a dive in March 1981 it was noted that large, filamentous "parachutes" were strongly luminescent when the water was perturbed by the motion of the submersible. This was observed only down to 108 cm, the top of the oxycline. Below that point the aggregates did not emit light. Marine snow is known to provide a habitat for very large numbers of bacteria (Caron et al. 1982), and the luminescence may therefore be of bacterial origin and hence oxygen dependent.

The *Pisces* can be set to move slowly forward at 0.5 knots ($25 \text{ cm} \cdot s^{-1}$), which allows the observers to note variations in plankton composition and density at any given depth. Patchiness was apparent in Saanich Inlet. In one dive, density of *Euphausia pacifica* at 104 m varied in a single transect from 1000 to $10\,000 \cdot m^{-3}$. *Aglantha digitale* varied from 2 to $25 \cdot m^{-3}$ at 100 m. Accurate plotting of patches should be feasible in a manoeuverable submersible such as *Pisces*.

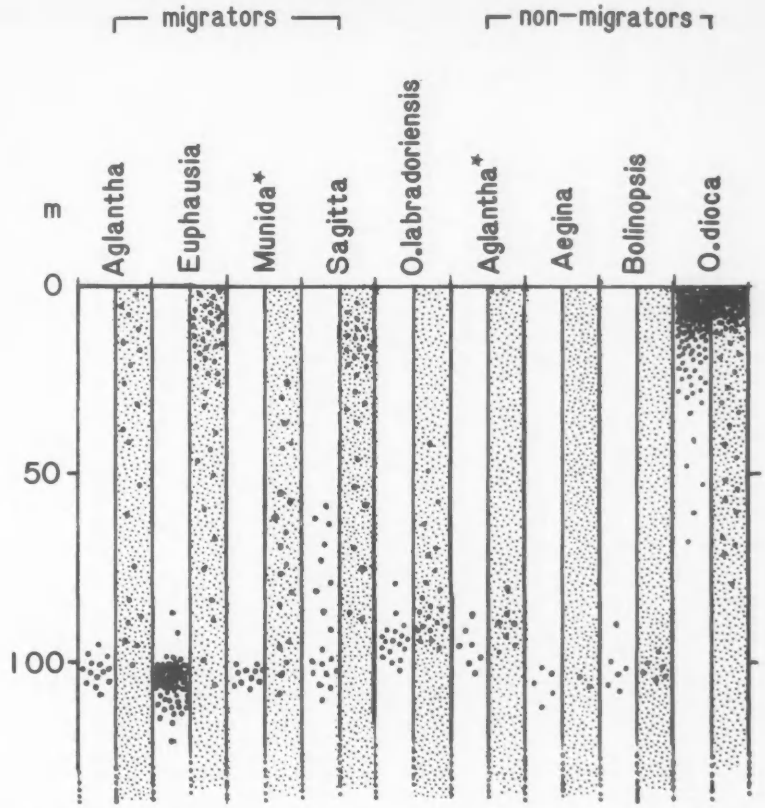

FIG. 7. Distribution of selected species as observed in consecutive day–night dives, station 2, Saanich Inlet, illustrating vertical migration. The day dive (empty columns) took place at 13:30–16:30 h, March 30, 1981, and the night dive (stippled column) took place at 21:00–23:00 h, March 30, 1981, except in the cases of *Munida** and *Aglantha**, which were observed during an earlier pair of dives on March 11, 1981.

TABLE 4. Stratification of *Euphausia pacifica*, Saanich Inlet, Aug. 1980–March 1981.

Data	Time	Station	Depth range (m)	Concentration depth (m)	Concentration density (No.·m^{-3})
Aug. 19–22	Day	C, D, E	120–170	147–150	1500
Nov. 4–7	Day	B, C	94–180	97–102	100–500
Mar. 11–12	Day	C	70–120	98–103	1000
	Night	C	10–105	10–40	500
Mar. 30–Apr. 3	Day	E, F	85–120	99–112	1000–10000
Mar. 30	Night	E	0–105	10–15	50

Discussion

BIOLOGICAL INSIGHTS

The current series of dives has allowed us to gain a better appreciation of the composition of the macroplankton communities along the coast of British Columbia. This applies especially to gelatinous forms (which were the main focus of the study) and most of all for those ctenophores poorly preserved in net hauls.

Data from net sampling (Hoos 1970), echo sounding (Bary 1967), and respiration measurements (Devol 1981)

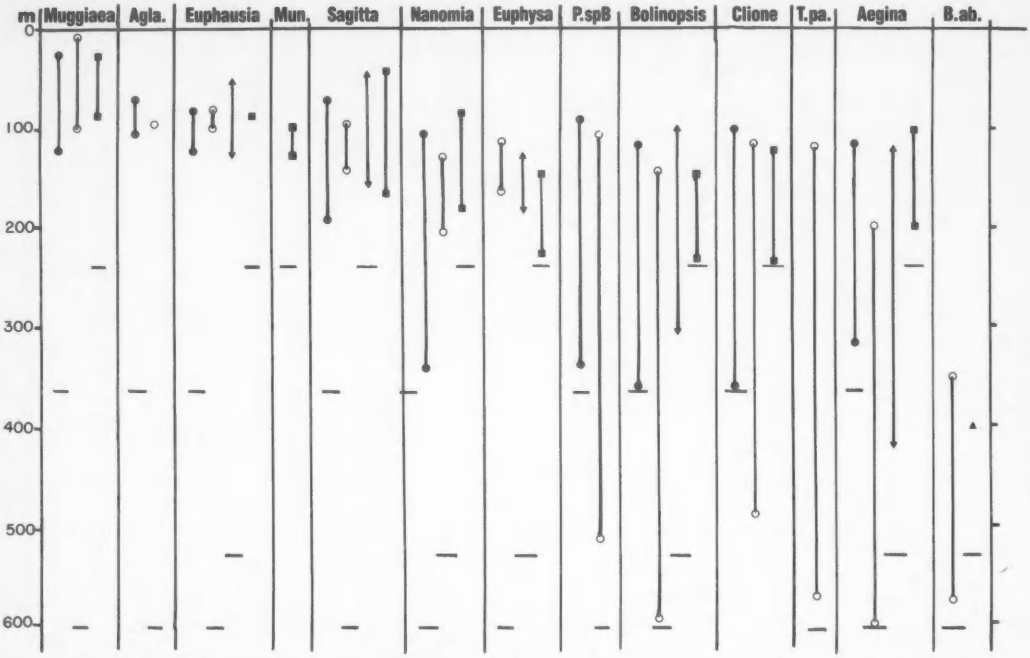

FIG. 8. Vertical distribution of selected species in Jervis Inlet, Knight Inlet, and Howe Sound, spring 1980 and 1981. Horizontal lines indicate depth to which submersible descended. ●, Jervis Inlet, May 21, 1981; ○, Jervis Inlet, February 22–23, 1981; ▲, Knight Inlet, February 12–19, 1981; ■, Howe Sound, March 17–20, 1981.

all indicate elevated concentrations of plankton over the oxycline, but the observations from *Pisces* show that several species (e.g. *Aglantha* and *Euphausia*) occur in much higher numbers and within much narrower bands than hitherto suspected. Waters containing less than about 0.2 mL $O_2 \cdot L^{-1}$ are known to be inhospitable to most zooplankton (Longhurst 1967) although where oxygen minimum layers show sufficient long-term stability some species may adapt toward a more anaerobic existence (Childress 1975), as *Orchomenella affinis* appears to have done in Saanich Inlet. The accumulation of plankton above the oxycline in Saanich Inlet may simply be due to a barrier or false-bottom effect upon diurnally migrating species (Devol 1981). The possibility that the abundance of zooplankton here is due in part to increased food availability is an attractive one, because microorganisms are known to thrive at oxic–anoxic interface regions in other areas (Karl et al. 1977). Bacteria catalyzing oxidation of manganese, iron, and sulphur could represent an important food source. The recent work of Devol (1981) shows no evidence of increase in microzooplankton respiration at the oxycline, but Emerson et al. (1979) estimate that 80% (by weight) of the particles in this zone are bacteria, and the issue cannot yet be regarded as settled.

Whether or not the high densities of euphausiids over the oxycline are partly due to their ability to exploit a chemoautotrophic carbon production, it is well demonstrated that they migrate to the surface at night and presumably ingest food derived from photosynthetic production. Several other crustaceans and *Sagitta* follow a similar migration path in Saanich Inlet. Information on copepods and other smaller crustacean zooplankters is scanty. These forms are difficult to observe from submersibles, but this drawback can be offset by better training of observers. Recently, we have found it possible to distinguish six copepod species from the observer ports of the *Pisces*.

We have verified that the dysaerobic zone of Saanich Inlet is inhospitable to all plankton, except the amphipod *Orchomenella affinis*. While Saanich is certainly a "rather weird" inlet (Pieper, in discussion following Bary and Pieper 1970), it has received a great deal of attention over the years since 1927 (Herlinveaux 1962) and is currently of interest in providing a possible model for the chemical–microbiological processes believed to be occurring in hydrothermal vent regions of the ocean floor.

POTENTIAL OF *PISCES IV* FOR PLANKTON WORK

The basic vehicle is one of the most manoeuverable small submersibles and is therefore well suited to delicate operations in the pelagic as well as in the benthic realms. The submersible can reveal important facts about both the composition and the distribution of the plankton and of the activities and interactions of the organisms composing it, adding to the data obtained by other means. There are, however, still some ways in which its usefulness could be improved, and some of these points may be applicable to other submersibles.

1) Training of observers: the ability of observers to distinguish as many organisms as possible down to the species level is a factor of the first importance and justifies extensive predive preparation.
2) Continuous automatic monitoring during dives of water conductivity, temperature, and depth, as well as oxygen content would be of considerable benefit in terms of the efficient use of observer time.
3) Light penetration: interpretation of plankton movements in the epi- and meso-pelagic realms obviously calls for exact information on light penetration.
4) For many applications, it would seem desirable to make dives concurrently with echo sounding at various frequencies. The observers in the submersible can directly report on the composition of the scattering layers.
5) Of great value is the regular checking of submersible sightings against data obtained by net sampling. Observers in the submersible can see many things missed by net sampling and vice versa. It would be of considerable interest to conduct submersible observations in conjunction with surveys made with a sophisticated closing net system such as Bioness (Sameoto et al. 1980).
6) Of proven value in the hands of submersible users elsewhere have been devices for capturing and transporting specimens undamaged to the surface and for obtaining seawater samples containing particles or other suspended objects under the direct control of observers within the submersible. Such devices should be developed for use with *Pisces*.

Acknowledgments

We are most grateful to the Director and staff of the Institute of Ocean Sciences, Patricia Bay, Victoria, for use of the *Pisces IV* and especially to the chief pilot, Frank Chambers, and pilots of the submersible itself. The observations from Jervis Inlet were made possible by an invitation from V. J. Tunnicliffe to participate in her dives in that locality. Dr Tunnicliffe also provided plankton data from Knight Inlet and Howe Sound. R. H. Herlinveaux, J. Fulton, and M. Youngbluth read and commented on the manuscript. Colleagues at the University of Victoria most directly involved in our work were Ron Larson and Patrick Kerfoot. Jeanette Yen, University of Washington, Seattle, provided valuable help with copepod spottings. The work was supported by a strategic grant from the Natural Sciences and Engineering Research Council of Canada.

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Embryo Movements of Atlantic Salmon (*Salmo salar*) as Influenced by pH, Temperature, and State of Development

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PETERSON, R. H., AND D. J. MARTIN-ROBICHAUD. 1983. Embryo movements of Atlantic salmon (*Salmo salar*) as influenced by pH, temperature, and state of development. Can. J. Fish. Aquat. Sci. 40: 777 – 782.

Two types of embryo movements of Atlantic salmon (*Salmo salar*), pectoral fin flutter and trunk movements, were measured as a function of incubation temperature, pH, and developmental state. Trunk movements began at 200 degree-days, initially at highest frequency. The frequency decreased throughout subsequent development and then increased slightly just before hatching. Movements were much more stereotyped in the earliest stages of development than just prior to hatching. Frequencies at 200 degree-days were insensitive to temperature and pH. Frequencies just prior to hatching were very temperature sensitive (Q_{10} of 13) and were decreased at low pH. This decreased frequency may be related to documented effects of temperature and low pH on hatching. Pectoral fin movements were temperature sensitive (Q_{10} of 2) and pH insensitive. These movements were initiated at 350 degree-days of development and attained maximal frequency at 400 degree-days.

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Deux types de mouvements d'embryons de saumon atlantique (*Salmo salar*), les battements des nageoires pectorales et les mouvements du tronc, ont été mesurés comme fonction de la température d'incubation, du pH et du stade de développement. Les mouvements du tronc commencent à 200 jours-degrés, la fréquence maximale se produisant au début. Cette fréquence diminue par la suite pour augmenter légèrement juste avant l'éclosion. Les mouvements sont beaucoup plus stéréotypés dans les premiers stades de développement qu'immédiatement avant l'éclosion. Les fréquences à 200 jours-degrés ne sont pas influencées par le température et le pH. Immédiatement avant l'éclosion, les fréquences sont très sensibles à la température (Q_{10} de 13) et diminuent à un bas pH. Il est possible que ce ralentissement soit lié à des effets prouvés de la température et du bas pH sur l'éclosion. Les mouvements des nageoires pectorales sont sensibles à la température (Q_{10} de 2) mais ne le sont pas au pH. Les battements commencent à 350 jours-degrés de développement et atteignent une fréquence maximale à 400 jours-degrés.

Received September 27, 1982
Accepted March 4, 1983

Reçu le 27 septembre 1982
Accepté le 4 mars 1983

REPRODUCTIVE failure resulting from lake or stream acidification has frequently been implicated in the loss of fish populations (e.g. Jensen and Snekvik 1972; Beamish et al. 1975; Schofield 1976; Harvey 1980). The manner in which low pH affects reproduction and recruitment appears to be variable. Several investigations have demonstrated that hatching success may be reduced, or the time of hatching delayed, at low pH (Mount 1973; Runn et al. 1977; Peterson et al. 1980). Runn et al. (1977) in studies on the perch (*Perca vulgaris*) and Peterson et al. (1980) in studies with Atlantic salmon (*Salmo salar*) postulated that hatching might be delayed due to inhibition of the hatching enzyme at low pH. Subsequently, Haya and Waiwood (1981) indicated that hatching enzyme (chorionase) activity is reduced in eggs incubated at pH 4.5 relative to those incubated at pH 6.5. However, the hatching enzyme digests only the inner proteinaceous layer of the salmonid egg zona radiata (Bell et al. 1969). Successful hatching also requires that the outer mucopolysaccharide layer be physically ruptured, presumably by the movements of the embryo inside. The decrease in chorionase activity observed by Haya and Waiwood (1981) could be due to inhibition of enzyme release from the rostral glands, as well as inhibition of enzyme activity. In this regard, Hayes (1942) suggested that embryo movement may be required for enzyme release through a "smearing" effect. Physical change in the egg zona radiata also makes hatching more difficult at pH's of 4.0–5.0 (Daye and Garside 1979; Haya and Waiwood 1981).

Peterson et al. (1977) observed that the hatching process has a high Q_{10} (approximately 8) and that the frequency of

Printed in Canada (J7068)
Imprimé au Canada (J7068)

swimming movements of hatched alevins is highly temperature dependent with a Q_{10} of approximately 5 (Peterson and Metcalfe 1979). Our objective, therefore, was to analyze embryo movements to see if they were influenced by low pH and temperature in a way that could be related to hatching success.

We also observed the frequency and complexity of embryo movements throughout development to determine any developmental patterns that might occur.

Materials and Methods

Newly fertilized Atlantic salmon eggs from three different stocks were used in the experiments. One lot was obtained from the Deer Island, N.B., fish culture facility and were of Saint John River, N.B., origin. The other two lots were obtained from the North American Salmon Research Centre, St. Andrews, N.B., one lot of unknown ancestry and the other derived from Big Salmon River, N.B., stock. The eggs of Big Salmon River stock were used for observations of pectoral fin movements, while the other two stocks were used for experiments with trunk movements.

The newly fertilized eggs from the first and third lots were placed in incubation boxes with water at ambient temperature flowing through the boxes at 1 cm/min over a single layer of eggs. Eggs incubated at low pH received water that had been acidified with 5% reagent-grade H_2SO_4 delivered from a Radiometer® titrator assembly with negative-feedback control of pH. After acidification, the water was passed through two aeration chambers to drive off CO_2 evolved from the acidification process. The ambient water supply had a pH of 6.6–6.9, with Na, Cl, Ca, Mg, K, and SO_4 levels of 100, 100, 38, 15, 10, and 20 μmol, respectively. The bicarbonate level of the unacidified water supply was 100–120 μmol. Free CO_2 level in control and acidified water (after aeration) was about 15 mg/L, as measured with an Orion® CO_2 electrode.

Pectoral fin movements were counted under a dissecting microscope at a magnification of 11.5×, with the egg being observed in an aerated, temperature-controlled, static water bath adjusted to the appropriate pH with H_2SO_4. The egg was illuminated from the side with a standard microscope illuminator adjusted to its lowest power setting for 10 min prior to observation and while observations were performed. The light intensity falling on the egg was 2500–3200 lx.

Eggs were set in slight depressions drilled into the bottom of an observation chamber. Embryo trunk movements were then observed under a mounted hand lens at 2.5× magnification. Water flowed through the chamber at 120–150 mL/min at a chamber volume of 300 mL (10 × 15 × 2 cm deep). Water at the appropriate pH could be tapped into the observation chamber from reservoirs of water at the required pH. The chamber was illuminated from the side with a flashlight; the face of the flashlight was covered with electrical tape except for a 1-cm slit. The initial light intensity in the chamber varied from 250 to 500 lx, depending upon whether the egg was situated directly in the light beam or to one side. Fresh batteries were inserted in the flashlight immediately before each bout of observations, which lasted up to 50–60 min. Some reduction of light intensity occurred during the course of observations but no apparent effect on frequency of movement was observed. Eggs were illuminated in the chamber for 1 h prior to observations. The frequency with which embryos moved varied greatly, depending upon their stage of development. For active, early-stage embryos (200 degree-days), each egg was observed for 5 min; for inactive embryos near the point of hatching, the observation period was 1 h/egg. The room in which observations were made was dark except for the illumination used for observations. Units of pectoral fin and trunk movements will be defined when these movements are described in detail under Results.

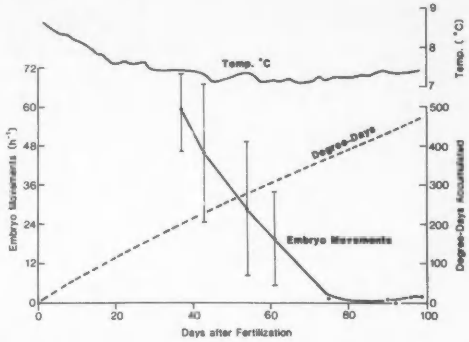

FIG. 1. Frequency of Atlantic salmon embryo body movements (±95% confidence limits) as a function of time from fertilization, 1980–81.

The influence of temperature on pectoral fin and trunk movements was investigated by passing water through the observation chambers from an aerated, thermostatically controlled, constant level reservoir. Temperature variation during experiments was within ±0.5°C of the desired temperature.

In some experiments, methylene blue was injected into the perivitelline fluid, using micropipettes inserted through the egg capsule. The dye injections were performed to determine the influence of embryo movements on the circulation of perivitelline fluid.

Results

Obvious trunk movements of embryos began abruptly at 200 degree-days of development (Fig. 1, 2), corresponding to about stage 17 in Ballard's (1973) anatomical classification. Prior to that point, they had not moved detectably. Movements at 250 degree-days were stereotyped, consisting of a flexure into a "C" shape with the nose and tail being brought into close proximity (Fig. 3). The flexure was followed by a slower relaxation until the embryo had once again straightened. A flexure in this early, stereotyped phase was considered to be a unit of activity. The frequency of flexing was relatively high initially (60–120 flexures/h) (Fig. 1, 2) and declined rapidly with further development until it averaged only 1 flexure every 2–4 h at 350–400 degree-days of development. Some 5–10 d prior to hatching, the activity increased to about 2 movements/h. At this point, movements

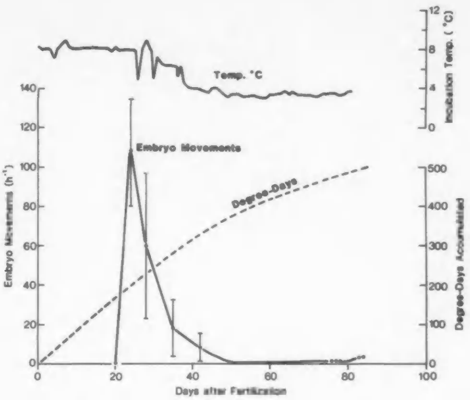

FIG. 2. Frequency of Atlantic salmon embryo body movements (±95% confidence limits) as a function of time from fertilization, 1981–82.

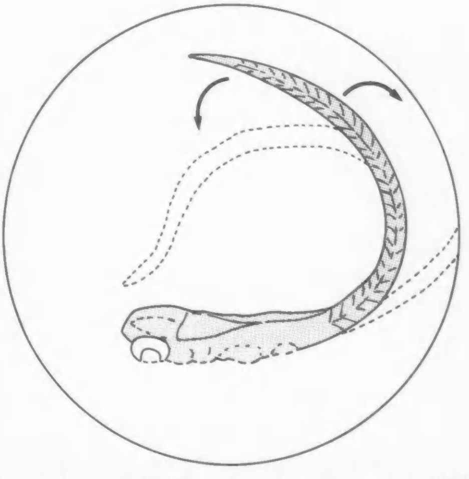

FIG. 3. Stereotyped body movements performed by Atlantic salmon embryos at 200–250 degree-days of development. The movement consists of a body flexure bringing the tail near the head followed by a slower subsequent extension to its former position.

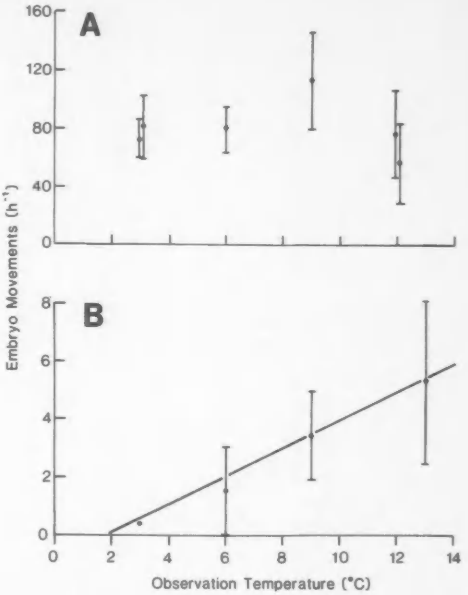

FIG. 4. Temperature dependence of Atlantic salmon embryo trunk movements in (A) the early, stereotyped phase and (B) the later phase, near hatching. Vertical lines indicate 95% confidence limits. No variation was found at 3°C in Fig. 4B.

were no longer as stereotyped and ranged from slight movements of the head or tail to "swimming" activity with the embryo and yolk revolving inside the zona radiata. In this advanced stage of development, any movement was considered a unit of activity. The duration of activity was measured in several experiments. The development of this increased complexity was not studied intensively because the embryos were often inactive for extended periods. Instead, observations in various pH and temperature regimes were made only for the two extremes of the spectrum: the active, stereotyped phase and the less active, more variable activity near hatching.

The frequency of stereotyped flexures of 250 degree-day embryos was not demonstrably temperature or pH dependent over the ranges of 3–12°C (Fig. 4A) and pH 4.5–6.5 (Table 1). The frequency of trunk movement of embryos near hatching (480 degree-days) is highly temperature dependent (Fig. 4B). The Q_{10} of activity over the temperature range 3–13°C is 13. The relationship is apparently linear, although the great variability in frequency of movement among embryos at the higher temperatures may mask the usual logarithmic relationship. The line of best fit is movement/h = $0.86 + 0.482T$ (°C) ($r = 0.55$).

At 3.0°C, 7 of 10 observed embryos were inactive for the hour of observation; at 6.0°C, 4 of 10 were inactive, while the proportions inactive at 9.0 and 13.0°C were 0 and 0.2, respectively.

Dye injection experiments indicated that trunk flexures result in rapid dye movements (Fig. 5B), primarily directed posteriorly along the flanks of the embryo. These flexures also resulted in movements of perivitelline fluid from one side to the other over the back of the embryo.

Exposure of eyed eggs to low pH (4.0–4.5) until hatching reduced the frequency of trunk movements near the time of hatching (Table 1) to about one-third of that of embryos incubated at control pH (6.7) (Table 1). Embryos at control pH moved an average of 1.7/h (1.3–2.0/h). Embryos reared at pH 4.0–4.5 moved an average of 0.64/h (0.25–0.85/h). Transferring embryos from pH 6.7 to 4.0 or 4.5 for 24 h was

TABLE 1. Frequency of trunk movements of Atlantic salmon embryos at two levels of development, reared at various constant pH levels.

Incubation pH	Test pH	Mean movements/h	95% confidence limits	N	No. hatched during time in observation chamber
Embryos at 240 degree-days (7.2°C test temp.)					
6.5	6.5	38	±14	11	—
4.5	4.5	37	±22	12	—
Embryos at 480 degree-days Exp. 1 (1980–81), 7.5°C (incubation and test temp.)					
6.7	6.7 (2 h)	1.8	±0.96	16	0
4.5[a]	4.5 (2 h)	0.25	±0.23	16	0
6.7	4.5 (24 h)	0.28	±0.71	7	1
4.5[a]	6.7 (24 h)	2.4	±2.0	8	0
Exp. 2 (1981–82), 3.5°C					
6.7	6.7 (2 h)	2.0	±2.0	8	0
4.0[a]	4.0 (2 h)	0.69	±0.64	16	0
6.7	4.0 (24 h)	0.67	±0.62	12	5
4.0[a]	6.7 (24 h)	1.8	—	4	5
Exp. 3 (1981–82), 6.0°C					
6.7	6.7 (2 h)	1.3	±0.47	26	3
4.5[a]	4.5 (2 h)	0.85	±0.32	27	0
4.5[a]	6.7 (24 h)	4.0	—	3	14
Exp. 1–3 combined					
6.7	6.7 (2 h)	1.7	±0.48	50	3[b]
4.0–4.5[a]	4.0–4.5 (2 h)	0.64	±0.22	59	0
6.7	4.0–4.5 (24 h)	0.53	±0.44	19	6[b]
4.0–4.5[a]	6.7 (24 h)	2.5	±1.1	15	19[b]

[a] Incubation pH after eye pigmentation; prior to eyeing, incubation pH was 6.7.

[b] Eggs at 480 degree-days were taken from lots where hatching was occurring. The numbers that hatched during observation are a function of test pH and time in test apparatus prior to observation (given in parentheses).

sufficient to reduce the activity to a mean frequency of 0.5/h, comparable with that of embryos incubated continuously at those low levels of pH. Conversely, transferring eggs from pH 4.0 or 4.5 to 6.5 resulted in an elevation in frequency of movement to 2.5/h, not significantly different from embryos reared continuously at control pH.

Sustained movement during a period of activity did not appear to be affected by exposing the embryo to low pH. The mean duration of a trunk movement was 2.1 s for embryos at pH 6.5 and 1.9 s for embryos at pH 4.0–4.5 (N = 53 and 22, respectively).

In one experiment with 10 embryos (incubated and tested at pH 4.5), the water supply failed during observations and the temperature rose from 5.0 to 9.0°C, possibly with an associated oxygen deficit. The embryos became very active, averaging 4.3 movements/h, yet none hatched during observation. Increasing the pH from 4.0–4.5 to 6.7 resulted in increased numbers of hatched alevins in the experimental apparatus (Table 1) during the 24-h period prior to observations.

During hatching of several embryos there was no movement of the embryo. The curled position of the embryo appeared to exert a tension on the zona radiata so that the egg was progressively deformed from its normal, spherical shape. Finally, the head of the embryo broke through the capsule. The entire process appeared to require little effort, and hatching was probably the result of the fragility of the zona radiata.

The first intermittent pectoral fin movements were observed at 69 d postfertilization (approximately 350 degree-days) (Fig. 6). This corresponds fairly well with the stage of development at which first fin twitching was observed by Ballard (1973) for rainbow trout (*Salmo gairdneri*) (stage 21B), although the degree-days differ considerably due to his higher incubation temperature (10°C). A unit of pectoral fin movement was considered to be one complete cycle (adduction plus abduction of the fin). The frequency of fin movement increased quickly until the rhythm was fully established at about 400 degree-days. The fin beat frequency, when fully developed, was temperature dependent with a Q_{10} of 2 from 2 to 12°C (Fig. 7). Maximal frequency attainable was 140–150/min and was generated by some of the embryos at 10–14°C. Embryos in eggs incubated at pH 6.5 from fertilization (380 degree-days) averaged 103.3 beats/min (N = 6, 95% confidence limits ±16.4), while those placed in pH 4.0 for 4, 14, and 16 d prior to observation averaged 109.3 ± 8.9, 115.7 ± 17.4, and 112.5 ± 9.2 beats/min when tested at 9°C.

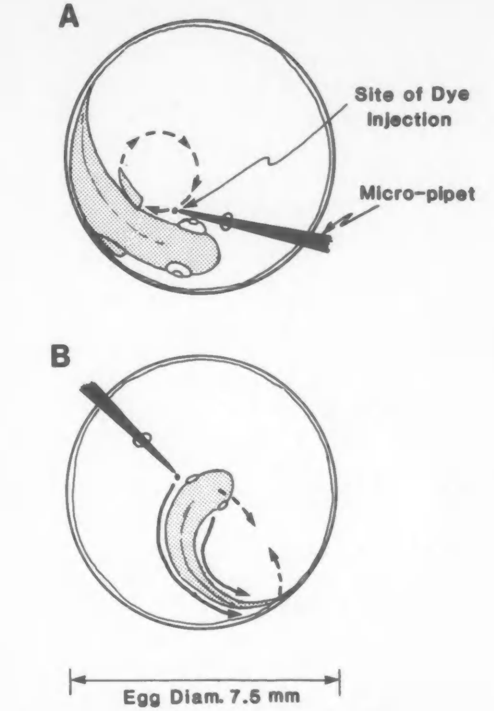

FIG. 5. Methylene blue trajectories in the perivitelline fluid resulting from (A) pectoral fin and (B) trunk movements of Atlantic salmon embryos. The dye was injected into the egg from micropipette. Arrows in Fig. 5A indicate movement of dye. Arrows with broken lines in Fig. 5B indicate the movement of the embryo that resulted in the dye movement (indicated by the arrows with solid lines).

A small amount of methylene blue, injected near the anterior edge of the pectoral fin, was propelled in a circular course by the fin movements (Fig. 5A). The diameter of the orbit was 2 mm and the mean time for completion of the circuit was 16.1 s (range = 13.1 − 18.4, $N = 4$) at 9.5°C. Little of the dye "leaked" from this circuit unless the embryo moved. None appeared to diffuse across to the other side of the embryo although, in two of the eggs, there was a tendency for some of the dye to diffuse posteriorly along the embryo's body. The perivitelline fluid set in motion by the pectoral fin movements, therefore, was confined mainly to the area adjacent to the embryo.

Discussion

These experiments have demonstrated that exposure of salmon embryos to low pH for the period from development of eye pigmentation to normal hatching can result in a reduction of the number of trunk movements at the time when hatching normally occurs. This movement reduction may contribute to the delayed hatching of embryos at low pH, either through reduced release of hatching enzyme or through less rapid rupture of the vestiges of the zona radiata.

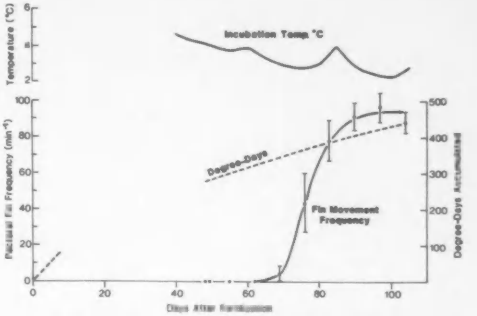

FIG. 6. Onset and frequency of Atlantic salmon embryo pectoral fin movement (±95% confidence limits) as functions of time from fertilization, incubation temperature, and thermal units accumulated.

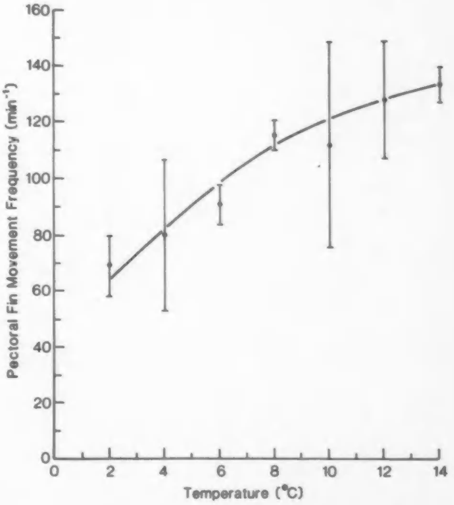

FIG. 7. Pectoral fin movement frequencies of Atlantic salmon embryos (±95% confidence limits) at 450 degree-days of development at various experimental temperatures. The curve was fitted by eye. Vertical bars indicate the 95% confidence limits.

The frequency of pectoral fin movements was not changed by exposing the eggs to low incubation pH. Peterson (1975) showed that pectoral fin frequencies in hatched alevins could be increased by depressing ambient $P\text{O}_2$. If this behavioral characteristic is respiratory in function, then changes in blood $P\text{O}_2$ or $P\text{CO}_2$ could be the stimuli responsible for modifying fin frequency.

The two types of physical activity monitored in these salmon eggs follow definite and consistent patterns during embryonic development (Fig. 8). The pectoral fin flutter and,

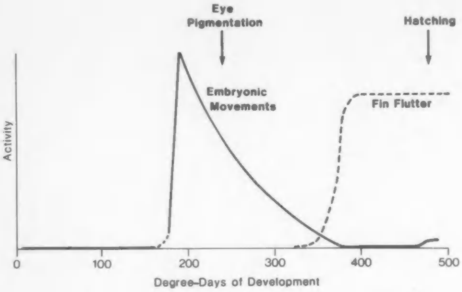

FIG. 8. Summary of the time course of development of the movements of Atlantic salmon embryos as a function of accumulated thermal units. Appearance of eye pigmentation and occurrence of hatching are also indicated.

in particular, the early trunk movements, appear within a short period of time (10 d for the former, <5 d for the latter). Balon (1979) developed the idea that ontogenetic processes are frequently saltatory rather than gradual. The two behavioral patterns described here seem to fall into this classification. Saltation may be the mode for the onset of specific behavioral patterns.

The function of the early trunk movements is unknown, as is that of the decline in frequency of movements. One hypothesis, which appears testable, is that the myotomes are initially not innervated and that frequency of activity declines as inhibition of spontaneous activity by neuronal invasion becomes more efficient. Alternatively, this activity could be a function of the state of development of controlling centers in the central nervous system. The initiation of pectoral fin flutter may also be generated as a result of development of appropriate neuronal centers. The primary function of pectoral fin flutter is probably respiratory, as it may facilitate movement of dissolved gases through the perivitelline fluid.

The pectoral fin flutter has a temperature dependence that is typical of many biological functions with a Q_{10} of 2 over the range of 2–12°C. This is a logical relationship for respiratory functions, as metabolic reactions tend to have a similar temperature dependence. Above 12°C, the rate may become temperature independent as maximal frequencies are attained.

The high Q_{10} (13) associated with trunk movements for embryos about to hatch is similar to that observed for frequency of movements of hatched alevins (Peterson and Metcalfe 1979). This high Q_{10} may be an adaptation allowing alevins to hatch quickly in response to increased temperatures in natural redds, a condition that would coincide with falling water levels (hence, decreased water percolation through the redd) and lower dissolved oxygen levels. These conditions would pertain to the postfreshet period in the spring when water levels are declining and water temperatures are increasing. These later trunk movements, unlike those of early embryos, seem highly adaptable to environmental change, as evidenced by their responsiveness to changes in temperature and pH.

Acknowledgments

F. Cunningham prepared the figures. K. Haya and R. Elner criticized the manuscript.

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Respiratory Adaptations of the Fingernail Clams *Sphaerium occidentale* and *Musculium securis* to Ephemeral Habitats

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Experiments performed on seasonal collections of the pisidiids *Sphaerium occidentale* and *Musculium securis* from a temporary pond suggest that respiratory adaptations are related to the specific life histories of the two species. *Sphaerium occidentale* lives up to 3 yr and all age classes aestivate during dry periods, while *M. securis* lives for about 1 yr and only newborn aestivate. Both species are iteroparous and reproduce primarily in late spring and early summer. Oxygen uptake rates at field temperature and Q_{10} values are low in both species during hibernation and aestivation, indicating respiratory stability and energy conservation. These rates and respiratory coefficients peak in spring during maximal growth and reproduction. Respiration in *M. securis* is more independent of temperature than in *S. occidentale*, reflecting the requirement of the former to complete growth and reproduction more quickly. Both animals show temperature acclimation patterns permitting early activity in spring and, thus, a more efficient exploitation of the short aquatic season. This pattern is more pronounced in the shorter lived *M. securis*. During aestivation, *M. securis* is a facultative anaerobe, while *S. occidentale* is an obligate aerobe. Neither species displays an oxygen debt following aestivation when immersed in water. Arousal from aestivation, as indicated by oxygen uptake, is delayed when clams are introduced to pond water. This apparently inhibits a premature resumption of activity and decreases the likelihood of desiccation in the natural habitat.

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Des expériences menées sur des échantillons saisonniers des pisidiides *Sphaerium occidentale* et *Musculium securis* d'un étang temporaire semblent indiquer que les adaptations respiratoires sont liées aux cycles biologiques spécifiques des deux espèces. La durée de vie de *Sphaerium occidentale* peut aller jusqu'à 3 ans, et toutes les classes d'âge estivent durant les périodes de sécheresse, alors que *M. securis* a une durée de vie d'environ 1 an, et seuls les nouveaux-nés estivent. Les deux espèces sont itéropares et se reproduisent surtout à la fin du printemps et au début de l'été. Chez les deux espèces, les taux d'absorption d'oxygène à la température ambiante et les valeurs de Q_{10} sont faibles durant l'hivernage et l'estivage, signes d'une stabilité de respiration et conservation d'énergie. Ces taux, ainsi que les quotients respiratoires, atteignent un sommet au printemps, en période de croissance et de reproduction maxima. La respiration chez *M. securis* est plus indépendante de la température que chez *S. occidentale*, ce qui reflète le besoin de la première espèce de compléter plus rapidement sa croissance et sa reproduction. L'acclimatation à la température chez les deux mollusques est telle qu'ils sont actifs tôt au printemps, exploitant ainsi plus efficacement la courte saison aquatique. Cette caractéristique est plus prononcée chez l'espèce à courte durée de vie, *M. securis*. Durant l'estivation, cette dernière est un anaérobie facultatif, tandis que *S. occidentale* est un anaérobie obligatoire. Une fois immersée dans l'eau, aucune espèce ne montre de dette d'oxygène après l'estivation. La sortie de l'estivation, comme l'indique l'absorption d'oxygène, est retardée quand les mollusques sont introduits dans l'eau de

Printed in Canada (J7122)
Imprimé au Canada (J7122)

l'étang. Ceci, semble-t-il, bloque une reprise prématurée d'activités et diminue la probabilité de dessiccation dans l'habitat naturel.

Received November 16, 1982
Accepted March 11, 1983

Reçu le 16 novembre 1982
Accepté le 11 mars 1983

PISIDIIDAE are cosmopolitan and ubiquitous in freshwater habitats. Many species inhabit temporary aquatic habitats and survive dry periods by aestivating. *Sphaerium occidentale* (Prime) is the only pisidiid found exclusively in ephemeral habitats (Herrington 1948, 1962). *Musculium securis* (Prime) inhabits lakes, streams, and both temporary and permanent ponds.

Sphaerium occidentale and *M. securis* have adopted appropriate life history strategies to withstand the environmental regimes of ephemeral ponds (McKee and Mackie 1981). *Musculium securis* has a 1-yr life cycle and only survives dry periods as newborn. This species grows rapidly to complete its life cycle before the pond dries. Each generation of *M. securis* grows synchronously, and relatively few length classes are present at any time. *Sphaerium occidentale* grows more slowly but aestivates at all sizes and survives for about 3 yr. Growth in both species occurs mainly in spring and early summer, although some growth also occurs in fall if surface water accumulates. Neither species grows in winter. Both clams show increased fecundity by producing large numbers of small newborn and by following iteroparous and multivoltine tactics. Increased fecundity counteracts density-independent mortality caused by desiccation (McKee and Mackie 1980). These species appear to demonstrate a mixed tactic response, according to the stochastic theory, for survival in highly variable environments (Stearns 1976, 1977).

The seasonal respiratory patterns of ectotherms may be used to indicate activity level, which in turn may be related to their specific ecologies. Several authors have related respiration rates to the specific ecology of pisidiids (Berg et al. 1962; Berg and Jonasson 1965; Alimov 1965, 1975; Collins 1967; Johnson and Brinkhurst 1971; Burky and Burky 1976; Holopainen and Ranta 1977a, 1977b; Waite and Neufeld 1977; Way et al. 1981); however, only Burky and Burky (1976) and Way et al. (1981) studied seasonal metabolic patterns. One objective of this study is to relate respiratory patterns of *S. occidentale* and *M. securis* to seasonal activity and environmental constraints in temporary ponds.

During aestivation, pisidiids must maintain tightly adducted valves to keep air, which may lead to desiccation and death, from the mantle cavity (McKee and Mackie 1980). Thus, respiratory tissues in *S. occidentale* and *M. securis* are isolated from atmospheric oxygen during dry periods. Respiration during aestivation is anaerobic in some molluscs (e.g. Meenakshi 1956, 1957) and is aerobic in others (e.g. Burky et al. 1972). Because pisidiids are ovoviviparous (Mackie 1978) and broods continue to develop in *S. occidentale* during aestivation (McKee and Mackie 1981), the growth of embryos and larvae may exert a metabolic requirement for oxygen. Owing to the lack of information on respiration during aestivation in *S. occidentale* and *M. securis*, experiments were undertaken to determine whether these clams require oxygen or acquire an oxygen debt during dry periods.

Materials and Methods

All studies were undertaken using clams collected from an ephemeral pond near Guelph, Ont. (43°30′N, 80°15′W). The pond filled in spring during snowmelt and dried in late spring and early summer. All surface water disappeared by mid- to late summer. The pond accumulated some water in early autumn, and anoxic conditions prevailed under ice from December to March during this study. Further details on the pond are given by McKee (1979), McKee and Mackie (1979), and Mackie (1979).

To express respiration rates on the basis of dry tissue weight, allometric relationships between surface-dried live clam weight and shell-free dry tissue weight were developed and are described by McKee (1979). Following oxygen uptake experiments, weights of surface-dried live clams were measured using a microbalance.

OXYGEN CONSUMPTION MEASUREMENTS

Respiration chambers consisted of 5.5-mL blood sampling tubes prewashed in 1.2 mol $HCl \cdot L^{-1}$ and rinsed in distilled water. Oxygen uptake of 1 − 15 clams per chamber filled with filtered pond water was measured. Initial dissolved oxygen levels were at saturation. Stoppers were inserted to preclude air bubbles. One control chamber without clams was prepared for every three to five experimental chambers. Experimental temperatures were maintained (±0.1°C) in a refrigerated constant-temperature bath. Experiments were run over 1 − 5 h. After incubation, each chamber was inverted a few times to ensure homogeneity, and 3 mL for oxygen determination was withdrawn by a hypodermic syringe.

Oxygen levels were measured with a Clark-type polarographic electrode (Clark 1956; Radiometer type E5046) mounted in a thermostated cell (Radiometer type D616). Samples were injected by hypodermic syringe into the thermostated cell to contact the $P\text{O}_2$ membrane. An acid−base analyzer (Radiometer type PHM72) coupled to a $P\text{O}_2$ module (Radiometer type PHA932) was used to monitor dissolved oxygen. Calibration was achieved with a zero-oxygen standard of sodium sulfite in 0.01 mol borax $\cdot L^{-1}$. Readings using samples of air-saturated deionized water at experimental temperature were taken after every three to five experimental readings. Differences between oxygen readings of the saturated standard were attributed to a linear change in electrode sensitivity. Oxygen uptake of clams was taken as the difference between experimental and mean control oxygen concentrations.

This oxygen measurement technique showed good reproducibility and precision. Oxygen readings in 15 air-saturated standards at 2 and 24°C (range of experimental temperatures) showed a coefficient of variability of 0.1% in both cases. Controls of initially saturated filtered pond water after 1 − 5 h of incubation had coefficients of variability of 1% in each experiment (9 − 13 determinations in each case). Filtered pond water partially deoxygenated at 20°C by bubbling with N_2 gas and siphoned into respiration chambers showed coefficients of variability of 1.7% at 70% saturation and 17% at 50% saturation after 1 h of incubation at 20°C (15 replicates in each case). Because reproducibility declined sharply below 70% saturation, incubation of clams in respiration chambers was terminated before oxygen levels fell below 70% saturation.

SEASONAL RESPIRATION

Experimental clams were collected monthly except in February between October 1977 and August 1978. Clams from the flooded pond were collected by long-handled sieve (0.32-mm openings) and from the dry pond using a garden spade. Clams were transported at field temperature to the laboratory in pond water or soil. Bottom-water temperature was measured during collections and periodically between collections using a hand-held mercury thermometer.

Sphaerium occidentale were grouped into three length classes for respiration experiments: <3, 3−5.49, and >5.49 mm. Respectively, these represented lengths of clams without developing broods, with marsupial larvae but without extramarsupial larvae, and with extramarsupial larvae ready for birth (McKee and Mackie 1981).

Only one size class of *M. securis* (mean length ±1 mm) was used each month, except in July when respiration of both newborn and parent clams was measured. Details on monthly size frequency and reproduction were provided by McKee and Mackie (1981). *Musculium securis* averaged 2.3−2.7 mm in length during experiments undertaken from October to April. About 75% of these clams had embryos and 0−20% had marsupial larvae in any month. Experimental clams in May had a mean length of 4.1 mm with 70 and 40% of these containing embryos and marsupial larvae, respectively. In June and July, mean lengths were 6.1 and 6.5 mm, respectively, with most clams containing extramarsupial larvae, marsupial larvae, and embryos. Experimental newborn *M. securis* in July and August were about 1.8 mm long.

Oxygen consumption experiments were carried out within 24 h of collection. Before experiments, clams were held at field temperature in a constant-temperature incubator. Shells were wiped clean of organic debris with laboratory tissues. Because oxygen consumption may depend on nutritional state (Alimov 1965), clams were first deprived of food in filtered pond water for 15 h. This period was adequate for emptying of gut contents, as observed through the transparent shell. In August, aestivating clams were held in filtered pond water for 15 h before measurement of oxygen uptake. With each group of clams, oxygen consumption was measured at field temperature and then at 10 and 20°C. Respiratory quotients (Q_{10} values) were calculated as (mean O_2 consumption at 20°C)/(mean O_2 consumption at 10°C).

The number of animals per replicate and the total number of replicates varied monthly depending on length class, experimental temperature, and the availability of clams. Numbers of *S. occidentale* per chamber were 4−6 for clams <3 mm, 2−4 for clams 3−5.49 mm, and 1−3 for clams >5.49 mm. Numbers of *M. securis* ranged from 15 for newborn (1.5−2 mm long) to 1 for the largest clams. Replicates ranged from 5 for each length class of *S. occidentale* and 4 for *M. securis* in fall and winter to 10 from April to August for each species and length class.

Student's *t*-test was used to detect effects of temperature on monthly oxygen uptake in *M. securis*, while analysis of variance (factorial design) was used to detect effects of temperature and length class on monthly uptake in *S. occidentale*. When temperature effects were insignificant at 10 and 20°C ($P > 0.05$), Q_{10} was reported as 1.0. When interaction effects between length classes of *S. occidentale* and temperature were significant ($P < 0.05$), Q_{10} values were calculated for each length class. Conversely, when the length class−temperature interaction was not significant, a mean Q_{10} was reported for all *S. occidentale* length classes.

AESTIVATION AND ANAEROBIOSIS

Experiments were undertaken in July to determine whether *S. occidentale* or *M. securis* acquires an oxygen debt during aestivation and to determine whether the presence of water induces the termination of aestivation, as indicated by respiration rates. Collections of *S. occidentale* (3−5.49 mm long) and newborn *M. securis* were taken from flooded areas of the pond and were maintained in a 1.5-L dish of pond water and sediments for 6 d at 20°C. These clams were then held in filtered pond water for 15 h prior to measuring respiration rates. These served as nonaestivating control animals. Similar collections were taken from an area of pond bottom that had been dry for 3 wk. Half of each collection of aestivating *S. occidentale* and *M. securis* was held in filtered pond water at 20°C for 15 h, and remaining clams were kept in moist soil at 20°C. A second collection of aestivating, 3−5.49 mm *S. occidentale* was taken from the dry pond and clams were divided into six groups of 30. One group was held for 2 d and a second group for 4 d in 20°C filtered pond water. The remaining four groups were placed in "maintenance dishes" of water, pond soil, and dried willow leaves at 20°C, providing suitable conditions for growth (McKee and Mackie 1981). Each group was held in maintenance dishes for 1, 2, 4, or 8 d. Before measuring oxygen uptake, each group from maintenance dishes was held in filtered pond water for 15 h. Aestivating clams held in moist soil were cleaned with laboratory tissues before being placed into filtered pond water for oxygen uptake determinations. Oxygen consumption rates of active clams and of aestivating clams held for various periods in moist soil, filtered pond water, or maintenance dishes were then determined at 20°C. Ten replicates with 3 *S. occidentale* or 10 *M. securis* each were used for each experiment. In these experiments, analysis of variance and Duncan's (1955) multiple range test were used to compare respiration rates.

To determine whether oxygen is required by either species during aestivation, 10 aestivating clams of each species were placed into each of two 125-mL flasks with 20 g of moist pond soil. A vial with a few pellets of KOH was placed inside one flask to absorb CO_2. This flask was then gassed with N_2 through an air-tight two-hole rubber stopper for 1 h. Half-hour gassings were applied daily to ensure the maintenance of anoxia. The second flask served as a control. Both were held at room temperature for 1 wk. Viability was then determined by microscopic examination for heart beats (McKee and Mackie 1980).

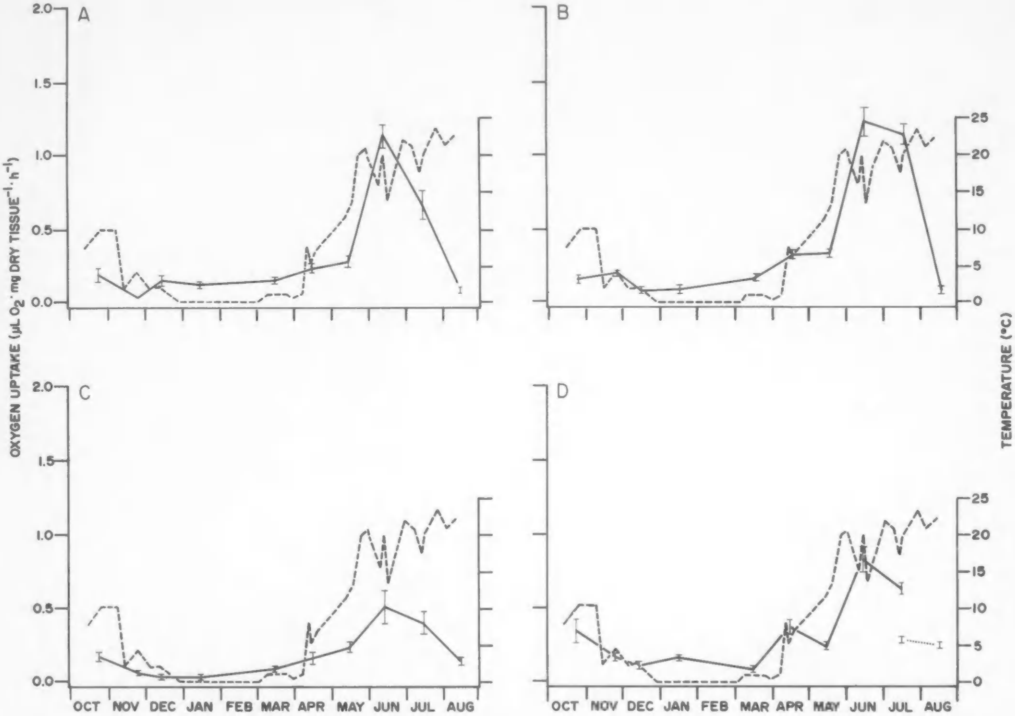

FIG. 1. Mean monthly oxygen uptake at field temperature for *S. occidentale* (A) <3 mm long, (B) 3–5.49 mm long, and (C) >5.49 mm long and for (D) *M. securis* parent generation (solid line) and newborn (dotted line). Broken line indicates field temperatures. Vertical bars show standard errors.

Results

SEASONAL RESPIRATION

Mean respiratory rates (microlitres of oxygen per milligram of dry tissue per hour) of *S. occidentale* at environmental temperature ranged from 0.033 to 1.134 for small clams (Fig. 1A), from 0.075 to 1.485 for medium-sized clams (Fig. 1B), and from 0.027 to 0.510 for large clams (Fig. 1C). Corresponding values for *M. securis* ranged from 0.090 to 0.836 (Fig. 1D). Lowest rates occurred in late fall and winter at low environmental temperature and during aestivation periods, while highest rates occurred in June at 20°C. Rates decreased slightly from October to winter as environmental temperatures fell from 10°C to freezing and increased slightly from March to mid-May as temperatures rose to 11.5°C. From May to June all respiration rates except those of the largest length class of *S. occidentale* increased dramatically by factors of 3.4–4.4. The corresponding increase shown by large *S. occidentale* was less than a factor of 2. At 20°C, aestivating *S. occidentale* in August displayed reduced aquatic respiration rates comparable with those exhibited in winter. The respiration rate of newborn *M. securis* in July was considerably lower than that of their parents. Aestivating newborn in August showed rates similar to the preaestivating newborn of July.

The oxygen consumption of field-acclimated *S. occidentale* at 10 and 20°C varied seasonally (Fig. 2A–C; Table 1). Weight-specific respiration appeared inversely related to clam size. There was no significant combined effect (*F*-test, $P > 0.05$) of temperature and length class on the respiration rates of *S. occidentale* from October to March when Q_{10} values ranged from 1.0 to 2.1. From April to June, respiration rates at 20°C generally increased compared with corresponding rates in winter, while those at 10°C were somewhat lower than rates in winter at this temperature. Respiratory quotients were highest in spring and summer, ranging from 1.0 to 4.3. During aestivation, temperature-specific respiration rates of all length classes were greatly reduced with Q_{10} values close to unity.

Respiratory responses of *M. securis* to 10 and 20°C also varied seasonally (Fig. 2D; Table 1). In late fall and early winter, Q_{10} values were 1.0 or less although from midwinter to early spring they increased to 1.7. From April to July, temperature-specific respiration rates fell and a Q_{10} of 2.1–2.5 was maintained. Oxygen consumption and Q_{10}'s of

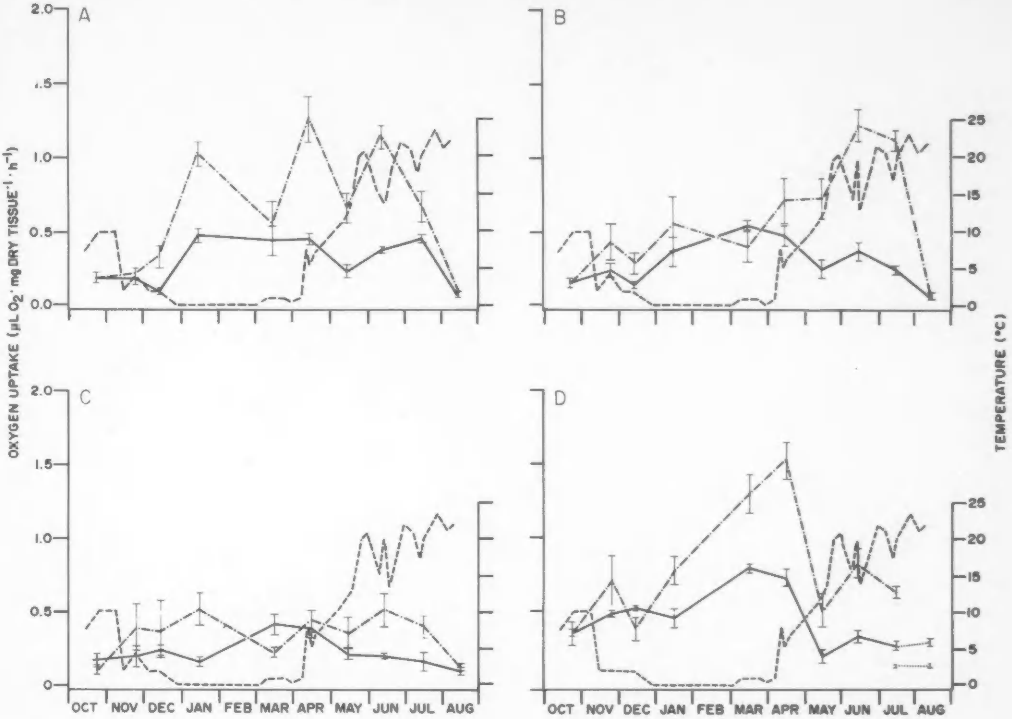

FIG. 2. Mean monthly oxygen uptake at 10 and 20°C for *S. occidentale* (A) <3 mm long, (B) 3–5.49 mm long, and (C) >5.49 mm long and for (D) *M. securis*. Solid line, uptake at 10°C; broken–dotted line, uptake at 20°C (*S. occidentale* and parent generation of *M. securis*); dotted lines Fig. 2D, uptake of newborn at 10°C (lower) and 20°C (upper). Broken line indicates field temperatures. Vertical bars show standard errors.

the newborn cohort were lower than those of their parents. Pre- and post-aestivating newborn displayed similar respiratory responses to temperatures with a Q_{10} of about 2.0.

TABLE 1. Monthly Q_{10} in three length classes of *S. occidentale* and of *M. securis* acclimated to field conditions. Mean lengths (mm) of *M. securis* are given in parentheses.

	S. occidentale length class (mm)			
Month	<3	3–5.49	>5.49	*M. securis*
October	1.0	1.0	1.0	1.0(2.3)
November	1.7	1.7	1.7	1.5(2.7)
December	2.1	2.1	2.1	0.7(2.7)
January	2.1	2.1	2.1	1.7(2.7)
March	1.0	1.0	1.0	1.6(2.7)
April	2.8	1.0	1.0	2.1(2.7)
May	2.8	1.0	1.0	2.5(4.1)
June	3.1	3.1	2.4	2.5(6.1)
July	1.0	4.3	2.3	2.3, 2.0[a](6.5, 1.8[a])
August	1.0	1.0	1.0	1.9[a](1.8[a])

[a] Newborn generation.

AESTIVATION AND ANAEROBIOSIS

No oxygen debt was acquired by *S. occidentale* during aestivation (Table 2). Oxygen uptake rates of aestivating clams in pond water for 0–4 d were lower than those of active control animals after 15 h in filtered pond water. After longer periods of food deprivation, *S. occidentale* exhibited respiration rates that did not differ significantly from rates displayed by aestivating clams in water (*F*-test, $P > 0.05$). Exposure to pond conditions in maintenance dishes for 1–8 d did not induce an increase in oxygen consumption in aestivating animals. This species, therefore, may require water or water and food for long periods (>8 d) before resuming normal activity after aestivation.

Musculium securis also showed no oxygen deficit during aestivation (Table 3). In contrast with *S. occidentale*, these clams resumed respiration rates similar to controls after 15 h in filtered pond water. Rates displayed by clams taken directly from pond soil were lower. Therefore, *M. securis* seems to require an aquatic environment for less than a day (but requires more than mere immersion in water) before resuming

TABLE 2. Mean oxygen uptake (μL $O_2 \cdot$mg dry tissue$^{-1} \cdot h^{-1}$) of *S. occidentale* at 20°C. Treatment 1: nonaestivating clams after 15–48 h in filtered pond water; treatment 2: aestivating clams after 0–48 h in filtered pond water; treatment 3: aestivating clams after 1–8 d in maintenance dishes. Ten replicates were used for each treatment and duration. Means followed by the same letter are not significantly different (Duncan's multiple range test, $P > 0.05$).

Treatment	Duration of treatment (h)	Mean oxygen uptake (standard deviations in parentheses)
1	15	0.733(0.441)
	48	0.228(0.069) ab
	96	0.282(0.182) ab
2	0	0.132(0.140) a
	15	0.202(0.111) ab
	48	0.221(0.158) ab
	96	0.156(0.114) a
3	24	0.150(0.017) a
	48	0.401(0.481) b
	96	0.281(0.009) ab
	192	0.268(0.135) ab

preaestivation rates.

All aestivating *M. securis* survived 1 wk in the N_2 atmosphere, while only 60% of aestivating *S. occidentale* remained viable. No deaths occurred among control clams aestivating in an aerobic environment.

Discussion

Just as smaller *S. occidentale* show high growth rates relative to larger individuals (McKee and Mackie 1981), young clams also display high respiration rates (Fig. 1A–C, 2A–C). Although large clams carry greater numbers and stages of larvae than do smaller animals, the respiratory demands of developing broods do not compensate for the low metabolic rates of their slow-growing parents. The continued development of litters during aestivation and the diminished rates of oxygen uptake at this time suggest that the impact of broods on oxygen consumption is small. In contrast, Burky and Burky (1976) found that over 50% of the oxygen consumption of *Pisidium walkeri* may be accounted for by developing litters.

While we did not determine the size–respiration relationship of *M. securis*, the sustained growth rates over a wide range of lengths (McKee and Mackie 1981) imply that respiration may be more independent of size in this species. The relationship showing newborn with much lower rates of oxygen consumption than their parents in July (Fig. 1D, 2D), should be attributed not to size, but rather to the dormancy assumed by *M. securis* at birth and the high activity levels of their parents.

The inverse relationship between size and weight-specific oxygen uptake observed in *S. occidentale* has been documented for other pisidiids (Alimov 1965, 1975; Johnson and Brinkhurst 1971; Holopainen and Ranta 1977a, 1977b). However, size-independent oxygen consumption has been reported in two species in this family (Burky and Burky 1976; Way et al. 1981). Burky and Burky (1976) discussed the potential effects of developing broods on the size–oxygen uptake relationship in *P. walkeri*.

Data on seasonal respiration rates at environmental temperatures (Fig. 1) corroborate results of life history studies (McKee and Mackie 1981), with the highest rates shown during periods of maximum reproduction and growth activities in *S. occidentale* and *M. securis*. Respiratory rates of large *M. securis* (mean length >6 mm) in June and July exceed those of large *S. occidentale* (>5.49 mm in length) by about 1.5 times, reflecting the requirement of the former species to sustain higher activity levels to complete its life cycle before the pond dries out. The lowest rates of respiration occur during dormancy in winter and in late summer. Intermediate rates of oxygen consumption in autumn reflect the clams' slow growth rates and reduced activities. In comparison, Burky and Burky (1976) reported minimum oxygen uptake in spring, maximum uptake in summer, and intermediate uptake in winter at field temperatures in *P. walkeri*. Way et al. (1981) observed a rapid increase in oxygen consumption during the period of peak growth and reproduction in spring, a decline in uptake over summer, and minimum consumption in winter at field temperatures in temporary and permanent pond populations of *Musculium partumeium*. These authors also related respiratory pattern to specific life history traits described in an earlier study (Way et al. 1980).

Changes in Q_{10} (Table 1) allow further interpretation of metabolic patterns. Low Q_{10} values of *S. occidentale* and *M. securis* during hibernation and aestivation probably function in dampening the effects of short-term temperature changes and in stabilizing metabolism. Homeostasis may be necessary to sustain populations during prolonged dormancy. Increases in metabolic activity induced by environmental fluctuations would otherwise deplete valuable energy stores which are nonrenewable during inactivity. Relatively high respiratory coefficients during late spring and early summer probably permit these animals to exploit good feeding condi-

TABLE 3. Mean oxygen uptake (μL $O_2 \cdot$mg dry tissue$^{-1} \cdot h^{-1}$) of *M. securis* at 20°C. Treatment 1: preaestivating clams; treatment 2: aestivating clams after 15 h in filtered pond water; treatment 3: aestivating clams placed directly into filtered pond water. Ten replicates were used for each treatment. Means for treatments 1 and 2 are not significantly different (Duncan's multiple range test, $P > 0.05$).

	Treatment		
	1	2	3
Mean oxygen uptake (standard deviation in parentheses)	0.297(0.036)	0.267(0.046)	0.139(0.046)

tions and to maintain high activity levels during the environmentally abbreviated growing season. Elevated Q_{10} values consequently allow the accumulation of energy stores by extramarsupial larvae of *M. securis* and by *S. occidentale* for consumption during aestivation. The maximum Q_{10} of *M. securis* is lower than those of *S. occidentale*, implying that the former species is more metabolically independent of temperature. Short-term fluctuations, therefore, affect the activity of *M. securis* less, and growth rates can be maintained at higher levels by this species at suboptimal temperatures, to the obvious advantage of these shorter lived animals. The retention of a low Q_{10} by both species in October indicates a continuation of the respiratory stability associated with diapause. *Sphaerium occidentale* and *M. securis* are like most poikilotherms in this respect, showing a lower Q_{10} when cold acclimated than when warm acclimated (e.g. Bullock 1955). *Pisidium walkeri* also displays seasonally varying temperature coefficients with a maximum Q_{10} in summer (Burky and Burky 1976).

A classification of the temperature acclimation types according to the system described by Precht et al. (1973) is informative. Increasing rates of oxygen uptake from fall to winter at 10 and 20°C (Fig. 2A–C) suggest a type I pattern of overcompensation in *S. occidentale*. This may act to stimulate activity quickly in spring with slight temperature increases, allowing these animals to lengthen their period of activity in spring and to compensate for the short aquatic season. The respiration rates of medium-sized clams from April to July at 20°C are greatly elevated above corresponding rates in winter, indicating a type V pattern of reverse compensation, while slight decreases in rates at 10°C during the same period suggest that a type I pattern is also followed. Large monthly fluctuations in uptake at 20°C exhibited by newborn *S. occidentale* make interpretation of the type of temperature acclimation difficult, although declines in rates at 10°C from April to May again suggest overcompensation at this temperature. Large *S. occidentale* display slight increases in rates at 20°C from March to June (type V), while respiration at 10°C decreases from March to May, indicating a type I pattern. Apparently, no single pattern of acclimation applies to field acclimated *S. occidentale* at all times of the year or at all temperatures. A type V pattern at high temperature (20°C) from winter to spring allows warm-acclimated clams to maintain higher rates of respiration (and increased exploitation of optimum growing conditions) than do other acclimation patterns. In the natural environment, this temperature is not reached until mid- to late May. At cooler temperatures (about 10°C) earlier in the season, a type I pattern prevents the more extreme increases in respiration rates that would result from the retention of the type V pattern. This may reflect an increase in efficiency of energy utilization and metabolic adjustments associated with the resumption of activity.

A more distinct type I pattern of overcompensation is shown by *M. securis* from fall through winter with respiration rates increasing markedly at 10 and 20°C with the progression of winter (Fig. 2D). This strategy is also probably important in triggering a quick response in these clams to rising temperatures, thereby offsetting the temporal restriction of the ephemeral aquatic habitat. The same overcompensation pattern is displayed from April to May when rates at 10 and 20°C decrease with increasing field temperatures. This is important for reducing the sensitivity of clams in early spring to warm temperatures and may reflect more efficient energy utilization and a major metabolic reorganization for the period of maximum activity.

In other pisidiids, Alimov (1965) demonstrated that the respiration of *Sphaerium corneum* at 20°C decreases from summer to fall, suggesting a type V pattern. Burky and Burky (1976) documented both reverse and overcompensation patterns in *P. walkeri* and attributed a type I pattern to more efficient energy utilization during growth and reproduction. Way et al. (1980), using data from Benjamin (1978), felt that temperature insensitivity during summer in *M. partumeium* may be associated with this species' apparent ability to respond to photoperiod as a cue for the initiation of growth and reproduction.

Anorexia may be a prerequisite for aestivation in pisidiids. The aquatic respiration rates of aestivating and food-deprived *S. occidentale* are indistinguishable after 2 d without feeding (Table 2). Preaestivating newborn *M. securis* have no discernible gut contents (McKee 1979), suggesting that anorexia and aestivation may also be associated in this clam. Because pisidiids are primarily filter feeders, the incompatibility of aestivation and feeding in this family is obvious. Anorexia also precedes aestivation commonly in snails (Russell-Hunter 1964).

The low rate of oxygen consumption in newborn *M. securis* relative to parent clams (Fig. 1D, 2D) has adaptive significance. Because *M. securis* growing beyond the newborn size class are unable to aestivate (McKee and Mackie 1980), the arrested growth, reduced activity levels, and concomitant low rates of oxygen uptake are vital in the preaestivation stage. When aestivating *M. securis* are placed directly into water, oxygen consumption levels are initially about half of those shown by preaestivating newborn or by aestivating newborn held first for 15 h in water. This suggests that oxygen uptake may be further suppressed in the absence of water. A decrease in gaseous exchange during respiration in the dry pond may minimize the risk of desiccation.

Knowledge of postaestivation oxygen uptake and survival in anaerobic environments provides insight into the physiology of these clams during aestivation. The absence of both an oxygen debt and an oxygen requirement in aestivating *M. securis* suggests an aerobic or nonglycolytic anaerobic metabolism during dry periods. Thus, *M. securis* is a facultative anaerobe during aestivation. Conversely, aestivating *S. occidentale* is an obligate aerobe, since this species dies under prolonged anoxic conditions. Thus, the absence of an oxygen debt during aestivation in this species is expected. The developing broods in *S. occidentale* during aestivation (McKee and Mackie 1981) may exert an energy requirement that is best satisfied by aerobic respiration. For a general discussion of biochemical adaptations to anoxia in Mollusca, the reader is referred to Hochachka et al. (1973).

Since aestivating *S. occidentale* die when deprived of oxygen, these clams must be capable of oxygen consumption with tightly closed valves. Moist and dry-surfaced inactive *S. occidentale* exhibit a reduced rate of oxygen uptake, equivalent to an average of 0.11 $\mu L\ O_2 \cdot mg$ dry tissue$^{-1} \cdot h^{-1}$ at 20°C (Collins 1967), which is similar to results found for aquatically respiring aestivating clams at the same temperature in this investigation (Table 2). Many intertidal bivalves are capable of aerial respiration by gaping their valves to expose respiratory tissue to the atmosphere and can tolerate some degree of desiccation (Newell 1973). Such behavior would be lethal to aestivating pisidiids, since these animals are out of water for much greater periods and 100% relative humidity is required for long-term survival (McKee and Mackie 1980).

Many or all pisidiids possess small perforations through their shells; these punctae are tubes within the calcareous layers formed by specialized mantle cells (pyramidal cells) of unknown function projecting into or through the shell (Mackie 1977). Although a comparative study was not done, one of the outstanding features of *S. occidentale* among Pisidiidae appears to be the great density of punctae on the shell imparting a "peppered" appearance. *Musculium securis* also possesses punctae, but their density appears to be considerably lower than in *S. occidentale*. Schroder (1907) believed the pyramidal cells in *Sphaerium* (*Musculium*) *lacustre* to function in shell secretion but later rejected this hypothesis and postulated a sensory function. Rosso (1954) also suggested a shell secretory role in *Sphaerium* (*Musculium*) *transversum*. Collins (1967) showed that in *S. occidentale*, most punctae are in the vicinity of the umbone and their numbers diminish gradually toward the shell margin. The areas of highest density are not regions of major shell reorganization; therefore, a shell secretory function is difficult to support. While a sensory function is possible, a respiratory function is also plausible. Collins (1967) showed that as these animals grow, the numbers of punctae increase so that the number relative to the entire surface area of the shell remains fairly constant at about $4.6-7.0 \cdot 0.09\ \mathrm{mm}^{-2}$. If pyramidal cells or the underlying mantle surface are responsible for oxygen uptake, there should be a direct correlation between surface area and oxygen use. Collins (1967) found this to hold true. The preponderance of punctae in the umbonal region nearest the heart also may reflect a respiratory function. Resultant advantages to the clam are obvious; during aestivation, oxygen could be absorbed when closed valves prevent branchial exchange and the animal could retain the advantages of aerobic respiration. Also, an aerobic metabolism could be maintained without active siphoning during periods of inactivity in water.

Aestivating *S. occidentale* do not resume normal respiration rates and activity even after 8 d in the presence of food and water (Table 2). Although these clams often open their valves and apparently "test" the new aquatic environment shortly after being placed in water, they soon close their shells and continue inactivity for several days. Newborn *M. securis* resume preaestivation respiration rates shortly after being placed in water (Table 3), although these rates still remain significantly reduced below those of their parents under similar conditions (Fig. 1D, 2D), suggesting a continuation of dormancy. The retention of lower respiratory coefficients by both species in October (Table 1) indicates a continuation of the respiratory stability associated with diapause.

Delays in the termination of dormancy are adaptively significant. Should a heavy, late-summer rainfall result in temporary puddles in the dry pond bed, aestivating clams might otherwise resume activity. Then valuable energy stores would be consumed when concentrations of food organisms would probably be low. As puddles disappear, clams would be required to resume aestivation with a smaller store of energy. A rapid disappearance of these puddles might prevent *S. occidentale* from reestablishing its apparent waterproofing described by McKee and Mackie (1980). Similarly, if *M. securis* newborn should become active and grow, then they would be incapable of successfully resuming aestivation. Therefore, the slow response to favorable conditions ensures that these animals will not be aroused prematurely from dormancy.

Acknowledgments

Mark Green drafted the figures. This study was funded by the Natural Sciences and Engineering Research Council of Canada, grant No. A-9882, awarded to G.L.M.

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Epilimnetic and Metalimnetic Primary Production in an Indiana Hardwater Lake

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KONOPKA, A. 1983. Epilimnetic and metalimnetic primary production in an Indiana hardwater lake. Can. J. Fish. Aquat. Sci. 40: 792–798.

Primary productivity in an Indiana lake, which contained a metalimnetic layer of cyanobacteria, was calculated for the summers of 1979–81 by using a numerical model. Production estimates of 183 and 187 g $C \cdot m^{-2}$ were obtained for 1979 and 1980; the estimate for 1981 was 50% higher. These values were two- to four-fold higher than estimates made in 1963 and 1964. Production in the metalimnion accounted for 29, 44, and 34% of the total during the three summers. Changes in the vertical distribution of biomass in the water column appeared to have had a greater effect upon metalimnetic production than differences in water transparency or incident solar radiation. The average values measured for the latter two variables were reasonably similar during the 3 yr, whereas the depth at which the metalimnetic layer of cyanobacteria stratified decreased from 9 m in 1979 to 5 m in 1981.

KONOPKA, A. 1983. Epilimnetic and metalimnetic primary production in an Indiana hardwater lake. Can. J. Fish. Aquat. Sci. 40: 792–798.

La productivité primaire dans un lac de l'Indiana contenant une couche métalimnétique de cyanobactéries a été calculée pendant les étés 1979–81 à l'aide d'un modèle numérique. Les estimations de production de 1979 et 1980 ont été de 183 et 187 g $C \cdot m^{-2}$, alors que celles de 1981 étaient de 50 % plus élevées. Ces valeurs sont de 2 à 4 fois plus grandes que les estimations de 1963 et 1964. Ces trois étés, la production dans le métalimnion a été responsable de 29, 44 et 34 % du total. Des changements de distribution verticale de la biomasse dans la colonne d'eau semblent influencer davantage la production métalimnétique que des différences de transparence de l'eau ou de radiations solaires incidentes. Les valeurs de ces deux dernières variables ont été raisonnablement identiques ces trois années, alors que la profondeur de stratification de la couche métalimnétique de cyanobactéries diminuait, passant de 9 m en 1979 à 5 m en 1981.

Received May 11, 1982
Accepted March 11, 1983

Reçu le 11 mai 1982
Accepté le 11 mars 1983

NORTHERN Indiana contains a number of marl lakes, which are characterized by high concentrations of bicarbonate and calcium ions, alkaline pH, and deposition of carbonate minerals (Wetzel 1966). These and other factors interact to limit productivity in such lakes (Wetzel 1972). Many of the lakes contain metalimnetic populations of phototrophs, which usually consist of diatoms, dinoflagellates, or cyanobacteria (Eberly 1964). The lake examined in this study, Crooked Lake, is a marl lake that contained dense cyanobacterial layers in 1979, 1980, and 1981.

Metalimnetic phytoplankton populations have been shown to exist in a number of different types of lakes (Eberly 1964; Fee 1978; DeAmezaga et al. 1973). However, the relative importance of metalimnetic populations to total lake productivity, and the factors that influence metalimnetic production, have not been well characterized. One might expect a significant fraction of the photosynthesis in the water column to occur in the metalimnion, because phototrophic population densities are often much greater than in the epilimnion. In this report, I use a numerical model to estimate primary productivity in Crooked Lake during the summers of 1979–1981 to examine the factors that influenced metalimnetic production. Calculation of primary production in the metalimnion, a region that has a temperature discontinuity and a physiologically distinct phytoplankton population from that in the epilimnion, presents computation problems not found in models that calculate production in a homogeneous epilimnion. These aspects are discussed for a model that is of general use in calculating metalimnetic production. Furthermore, the results are compared with primary production values determined in 1963–1964 (Wetzel 1965) and related to other changes found in Crooked Lake.

Printed in Canada (J6932)
Imprimé au Canada (J6932)

Materials and Methods

Crooked Lake is located in Noble and Whitley counties, Indiana, USA. Data were collected from the lake at weekly or biweekly intervals from April to September during 1979, 1980, and 1981. The water column was 30 m deep at the sampling site. Temperature and oxygen measurements at

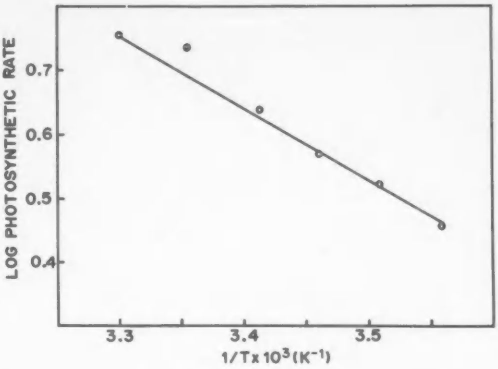

FIG. 1. Arrhenius plot of the maximum photosynthetic rate of Crooked Lake phytoplankton at different temperatures. The photosynthetic rate of a sample from the metalimnion was measured at 300 μE·m^{-2}·s^{-1} at temperatures from 8 to 30°C.

various depths of the lake were made with a YSI model 54 temperature–oxygen probe (Yellow Springs Instruments, Kettering, OH). Light penetration was determined with a Li-Cor quantum sensor (Lambda Instruments, Lincoln, NE). Water samples were collected with a Van Dorn bottle (Wildco Supply Co., Saginaw, MI). Chlorophyll *a* concentrations in samples were determined from the absorbance at 663 nm of dimethyl sulfoxide–acetone extracts (40:60) of organisms filtered onto glass fiber filters (Burnison 1980).

The photosynthetic rate of natural samples was determined at six light intensities at 10- to 14-d intervals during 1980 and 1981. Photosynthetic rate was measured in a sample from 2 m (epilimnion population) at irradiances up to 1500 μE·m^{-2}·s^{-1}. In a sample from the metalimnetic chlorophyll maximum, photosynthetic rate was determined at intensities of 0–300 μE·m^{-2}·s^{-1}. Experiments were initiated in the Crooked Lake Biological Station laboratory within 30 min after sample collection. Photosynthesis was determined by the uptake of $NaH^{14}CO_3$ into particulate material. An amount of 74 kBq of ^{14}C was added to 25 mL of lake water in a screw cap test tube. In the laboratory, tubes were incubated in duplicate at 25°C for 3 h at the appropriate light intensities using photoflood lamps for the epilimnion samples and cool white fluorescent lamps for the metalimnion samples. At the end of the incubation period, samples were filtered through either 0.45-μm pore size membrane filters (Gelman GN-6) for epilimnion samples or glass fiber filters (Reeve Angel 984H) for metalimnion samples, rinsed with distilled water, exposed to HCl fumes for 20 min, dried, and counted in a Tracor Delta 300 scintillation counter by employing a toluene-based scintillation cocktail with 2,5-diphenyloxazole (4 g·L^{-1}) and *p*-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (0.1 g·L^{-1}). The concentration of dissolved inorganic carbon was determined from measurements of pH and alkalinity (Stumm and Morgan 1970).

In situ measurements of primary production were made by collecting samples from discrete depths before 08:00 adding 74 kBq of $NaH^{14}CO_3$ to 25 mL of lake water in a screw cap tube. For each sample, three tubes (two light and one dark) were suspended at the depth from which the sample was collected. After incubation for 3–4 h, the tubes were recovered and processed as described above.

Solar radiation measurements were made during 1981 at the Crooked Lake Biological Station with a LiCor quartum sensor attached to an integrator (LiCor Solar Monitor, Lambda Instruments, Lincoln, NE). Solar radiation data for 1979, 1980, and 1981 were obtained from the U.S. National Weather Service station located in Ft. Wayne, IN, located 50 km from the study site.

A numerical model was used to calculate primary production. A program was written in Pascal 6000 and run on a Control Data Corporation 6500 computer at the Purdue University Computing Center. The program takes as input the vertical distribution of light penetration, temperature, and chlorophyll *a* in the lake, as well as the photosynthetic response of phytoplankton to light intensity measured in the laboratory. Solar radiation values may be input or calculated by the program.

Results and Discussion

Photosynthetic production at 1-m depth intervals and hourly time intervals was calculated according to the equation of Jassby and Platt (1976):

$$P(\mathrm{mg\ C\cdot m^{-3}}) = P_m^B \cdot \tanh\,(\alpha \cdot I/P_m^B)\cdot C \cdot T$$

where P_m^B and α are kinetic parameters corresponding to the maximum rate (milligrams of carbon per milligram of chlorophyll *a* per hour) and initial slope of photosynthetic rate as a function of light irradiance for natural populations. These parameters were determined from measurements of photosynthetic rate that were made in laboratory incubators. The light irradiance, *I*, at the depth and time considered, was calculated using vertical profiles of light attenuation in the lake coupled with values for solar radiation determined (1) mathematically for cloudless weather (Brock 1981), (2) by direct measurement at Crooked Lake, or (3) from U.S. Weather Service data collected 50 km away in Ft. Wayne, IN. Term *C* is the density of algal biomass (milligrams of chlorophyll *a* per cubic metre) and *T* is the time increment (hours). In lakes such as Crooked Lake, where the depth of the photic zone exceeds the depth of the epilimnion, there are two additional factors to consider in calculating production. First, the lake contains distinct phototrophic populations in the epilimnion and metalimnion. Photosynthetic responses to light irradiance were measured in samples from both strata. The second problem is that temperature within the metalimnion varied from 6 to 17°C and temperature changes could affect short-term photosynthetic rates and cause physiological changes in the metalimnetic phytoplankton species found at different strata. Experimentally, it was most convenient to measure the functional response of photosynthetic rate to changes in light intensity at one temperature (25°C) and then make corrections in the model for in situ temperatures. The effect of temperature upon short-term photosynthetic rates is primarily upon P_m^B rather than α (Aruga 1965; Li 1980; Konopka 1981). Experimental measurements of P_m^B at a

TABLE 1. Comparison of in situ measurements and model estimates of primary production in Crooked Lake for three types of weather conditions (data expressed as mg $C \cdot m^{-3} \cdot d^{-1}$).

	July 12, 1979, sunny		September 7, 1979, partly cloudy		October 6, 1979, cloudy	
Depth (m)	In situ	Model	In situ	Model	In situ	Model
0	50	70	112	152	25	40
1	61	88	113	135	23	39
2	92	47	114	122	20	28
3	63	58	85	92	20	24
4	68	74	56	69	16	20
5	84	71	56	43	10	13
6	123	70	37	69	10	11
7	68	110	65	47	6	7
8	817	1140	40	34	9	9
9	934	639	41	31	3	8
10	26	25	99	65	10	13
Total (mg $C \cdot m^{-2} \cdot d^{-1}$)	2566	2392	818	859	152	212

series of temperatures were made in samples from the metalimnion of Crooked Lake (Fig. 1), and a Q_{10} of 1.5 was found. This was similar to the values previously reported for organisms from this lake (Konopka 1981). In the numerical model, P_m^B was corrected for differences between the temperatures of the environment and the experimental incubators by using the Q_{10} value. Physiological changes that resulted from differences in the temperature at which metalimnetic organisms existed might also affect primary productivity. However, possible effects of temperature "adaptation" were ignored in this study for two reasons. First, most studies have found that although the photosynthetic rate per cell is different for organisms adapted to different temperatures, photosynthetic rate per unit of chlorophyll (as used in this study) did not change (Rhee 1982). Second, the gas vacuolate cyanobacteria found in Crooked Lake vertically migrated through the metalimnion (Konopka 1982a). Although their rate of movement was not fast (approximately 1 $m \cdot d^{-1}$), this vertical movement would prevent physiological heterogeneities in photosynthetic rates if it were faster than the time required for temperature "adaptation." This time course is poorly understood, and values from 0 to 100 h have been reported (Li 1980). Because of these considerations, and previous data that showed that organisms sampled from different temperatures within the metalimnion had the same photosynthetic rates (Konopka 1980), no corrections for physiological adaptations to temperature at different depths were made.

The two-parameter model was used to describe photosynthesis at different irradiances instead of a four-parameter model that included terms for high irradiances and dark respiration (Platt and Gallegos 1981). This latter model was not used because photoinhibition was not found in epilimnion samples incubated at irradiances up to 1500 $\mu E \cdot m^{-2} \cdot s^{-1}$, and light intensities in the metalimnion never exceeded 200 $\mu E \cdot m^{-2} \cdot s^{-1}$, well below the threshold at which photoinhibition was observed in metalimnetic populations.

Fee (1978) corrected the metalimnetic production values that were calculated using a numerical model to "force" them to agree with production values obtained from in situ incubations. Although he originally suggested that such corrections were necessary because of differences in light quality between the lake metalimnion and the laboratory incubators, it is now known that the problem was a systematic error in measuring irradiances in the incubators (E. Fee, Freshwater Institute, Winnipeg, Man., personal communication). This procedure was not used in this study, because the model becomes less general. These correction factors must be determined for each lake, and values for a particular lake will probably change during the season, because the light extinction coefficients and photosynthetic characteristics of the phytoplankton vary (Konopka 1982b). Whereas Fee (1978) reported that his model overestimated in situ production two- to five-fold in the metalimnion and hypolimnion, the differences in this study were much less (Table 1). Although I cannot explain the smaller differences between the model and in situ measurements in this study, metalimnetic samples were incubated under lamps that emit strongly in the 500- to 600-nm range, which has been found to be the most penetrating component of sunlight into the lake (R. Wetzel, Kellogg Biological Station, Michigan State University, Hickory Corners, MI, personal communication). For this study, it was preferable to have a small discrepancy between the model and measured in situ production rather than introduce factors for which there is no clear biological rationale.

Estimates of primary production were restricted to the periods May 1–August 31 of 1979, 1980, and 1981. Crooked Lake was thermally stratified at these times and contained a metalimnetic layer of cyanobacteria. Kinetic parameters for photosynthesis were determined for epilimnetic and metalimnetic samples during these periods in 1980 and 1981 (Table 2). These data were not collected in 1979, but to estimate production during this year the means of the parameters determined during 1980 and 1981 were used in the numerical model.

The most direct comparison of productivity among the 3 yr occurs if identical inputs of solar radiation are used. The intensity of cloudless solar radiation was calculated (Brock 1981) at hourly intervals for each day and used to estimate primary productivity (Fig. 2; Table 3). Total production was similar in 1979 and 1980 but 50% higher in 1981. Production in the top 4 m of the water column was twofold greater in 1981, primarily because a relatively dense population (16 mg

TABLE 2. Mean and standard deviation of photosynthetic parameters measured in samples from Crooked Lake.[a] (α = g C·mg chlorophyll $a^{-1}\cdot h\cdot(\mu E\cdot m^{-2}\cdot s^{-1})^{-1}$; P_m^B = g C·mg chlorophyll $a^{-1}\cdot h^{-1}$; N = number of determinations made from May 1 to August 31 of each year.)

	1980	1981
Epilimnion sample		
α	0.042±0.030	0.040±0.026
P_m^B	6.2±2.4	5.2±1.7
Metalimnion sample		
α	0.112±0.12	0.050±0.022
P_m^B	3.4±1.2	4.3±1.5
N	10	7

[a] In the numerical model, the kinetic parameters that were used to calculate production for a specific day were those experimentally determined on the date closest to the day for which production was calculated.

chlorophyll $a\cdot m^{-3}$) persisted in the epilimnion during May and contributed 40 g of C production, and chlorophyll *a* levels in the epilimnion during the remainder of the summer were approximately twice as large as in previous years. Total metalimnetic production (at depths below 5 m in 1979 and 1980 and depths below 4 m in 1981) was similar in 1980 and 1981, although metalimnetic production was a smaller proportion of the total in 1981 because of increased production in the epilimnion.

The production estimates listed in Table 3 represent an upper limit for actual production in the lake because they were calculated using maximum possible solar radiation. Actual daily radiation was estimated from data collected by the U.S. National Weather Service at Fort Wayne, IN. A linear regression of the daily percent possible sunshine reported by the U.S. National Weather Service against the quotient of daily quantum radiation measured at Crooked Lake divided by the calculated cloudless radiation for the day was determined for the period May 1–August 31, 1981 (r = 0.74). The regression coefficients were used to estimate daily quantum radiation for each year from U.S. National Weather Service data. Production values calculated using these estimates of solar inputs were 170.2, 177.4, and 246.2 g $C\cdot m^{-2}$ for May 1–August 31 of 1979, 1980, and 1981, respectively. These values are very similar to those calculated using cloudless insolation, except for 1981, in which the estimate was 10% lower. Radiation measured by the U.S. National Weather Service was proportionally lower in 1981; the mean value of possible sunshine decreased from 81% during the summers of 1979 and 1980 to 73% in 1981. Solar radiation at Crooked Lake was directly measured during 1981, and using these data, a value of 228 g $C\cdot m^{-2}$ was calculated for the period May 1–August 31, 1981. Therefore, the use of U.S. National Weather Service radiation data in this study may have overestimated production by about 10%.

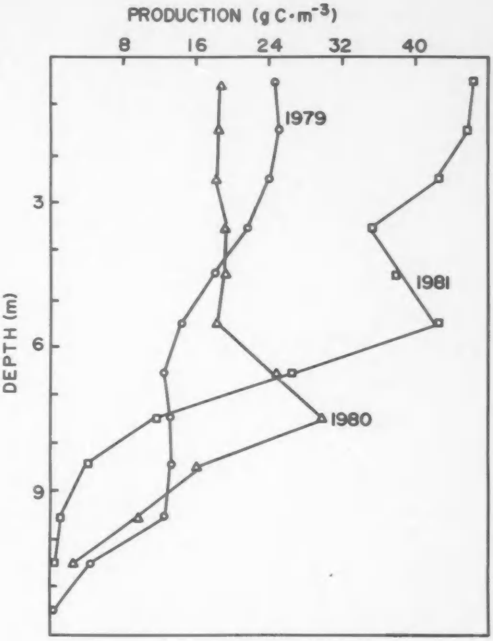

FIG. 2. Photosynthetic production at 1-m depth intervals in Crooked Lake for the period May 1–August 31 of 1979 (○), 1980 (△), and 1981 (□). Production was calculated using cloudless solar radiation as the input to the numerical model.

TABLE 3. Primary productivity and some environmental parameters of Crooked Lake.

Year	Production[a] (g $C\cdot m^{-2}$)	Metalimnetic production[a] (g $C\cdot m^{-2}$)	Light extinction coeff·(m^{-1})[b]	mg chl $a\cdot m^{-2}$[b]	Depth of metalimnetic layer (m)[c]	% surface light	N[d]	Predominant species
1979	183	53.4	0.50±0.15 (0.34–0.67)	169±53 (97–267)	9 (7–11)	1.1	12	*Oscillatoria rubescens*
1980	194.6	86.4	0.54±0.05 (0.43–0.59)	99±29 (69–159)	7 (6–8)	2.3	15	*Oscillatoria rubescens* *Oscillatoria redekei*
1981	295	99.4	0.60±0.19 (0.42–0.89)	120±30 (73–169)	5 (4–6)	8.2	14	*Aphanizomenon flos-aquae* *Oscillatoria rubescens*

[a] Calculated for May 1–August 31, presuming cloudless solar radiation.
[b] Values are reported as annual mean ± standard deviation, with annual range listed in parentheses.
[c] Values are reported as median, with annual range listed in parentheses.
[d] Number of vertical profiles measured from May 1 to August 31.

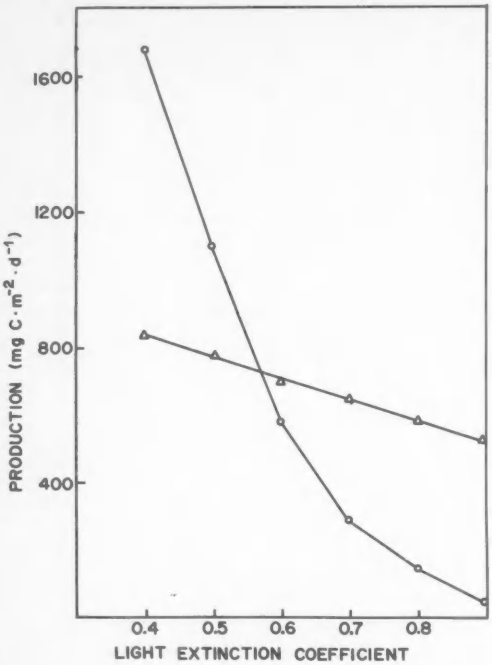

FIG. 3. Simulation of primary productivity in the epilimnion (△) and metalimnion (○) at different values of the light extinction coefficient into the water column. Other factors for the simulation were average values measured in 1980, and solar input of 50 E·m^{-2}·d^{-1} was specified.

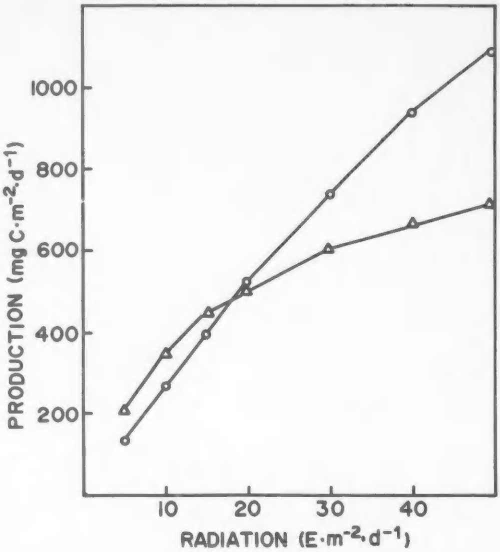

FIG. 4. Simulation of primary productivity in the epilimnion (△) and metalimnion (○) at different daily solar radiation input. Other parameters for the simulation were average values measured in 1980.

In 1963 and 1964, the primary productivity of Crooked Lake was determined using in situ incubation of samples to which NaH^{14}CO$_3$ was added (Wetzel 1965). For the period May 1–August 31, production was 112 g C·m^{-2} in 1963 and 70 g C·m^{-2} in 1964. These values are two- to four-fold lower than those calculated for 1979–1981. Thus, primary production in the lake appears to have increased during this time interval, although it is difficult to know how much the different methods used to estimate production, rather than real increases in algal production, contributed to the observed increases.

The concentration and photosynthetic activity of phytoplankton are important determinants of primary productivity. However, the relative importance of productivity in the metalimnion is determined by the intensity of solar radiation, transparency of the water column, and depth distribution of the metalimnetic population, all of which affect the light intensity that reaches the phototrophs in the metalimnion. Computer simulations were used to study each factor independently; typical values found in 1980 were specified for other parameters used in the calculations. Changing the light extinction coefficient had the most severe effect upon metalimnetic production (Fig. 3). However, the light extinction coefficients measured in the lake were fairly consistent from June through August; most of the dispersion in these data was due to high coefficients measured in May. Furthermore, although the mean light extinction coefficient increased from year to year, the metalimnetic production estimate also increased. Thus, variation in light transparency was not the most important factor affecting production in the metalimnion.

Metalimnetic production decreased more sharply than photosynthetic production in the epilimnion when the amount of solar radiation entering the lake was decreased (Fig. 4). Thus, cloudy climatic conditions would diminish the relative importance of metalimnetic production, but if such conditions persisted for several days, the gas vacuolate cyanobacteria could compensate by floating up to a shallower depth. No clearcut correlations between the depth of cyanobacterial stratification and changes in radiation input were found in Crooked Lake. However, the conditions under which this response was anticipated (very low solar radiation inputs) only persist for 2–3 d, and the cyanobacterial filaments can only migrate 1 m in this time (Konopka 1982a).

Variations in the depth at which the metalimnetic phytoplankton stratified had the greatest actual effect upon metalimnetic production in the lake. Computer simulations demonstrated that shifting the metalimnetic layer of cyanobacteria 1 m toward the surface doubled the calculated production in the metalimnion. Total phototrophic biomass (chlorophyll *a* per square metre) in the water column was larger in 1979 than in subsequent years, but metalimnetic production was much less that year because most of the biomass consisted of *Oscillatoria rubescens*, which stratified at

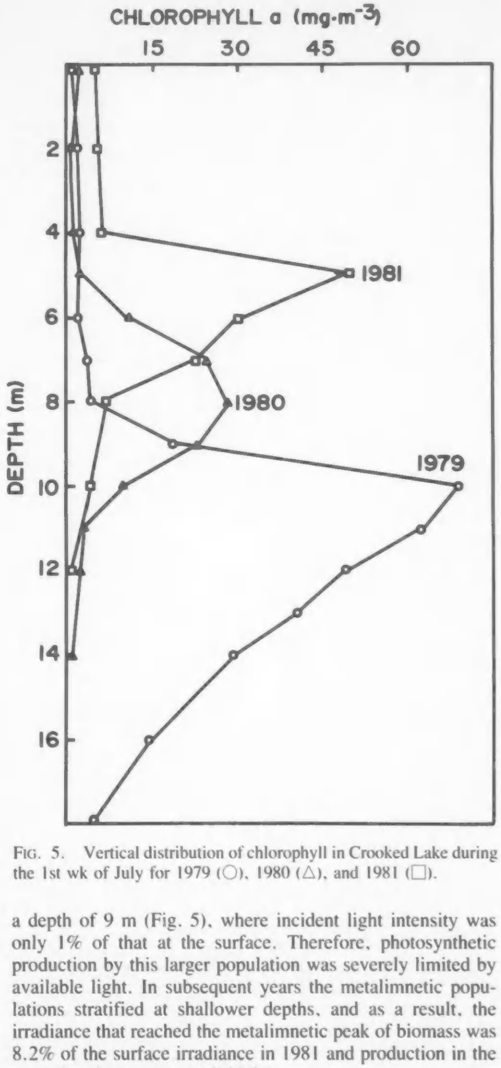

FIG. 5. Vertical distribution of chlorophyll in Crooked Lake during the 1st wk of July for 1979 (○), 1980 (△), and 1981 (□).

a depth of 9 m (Fig. 5), where incident light intensity was only 1% of that at the surface. Therefore, photosynthetic production by this larger population was severely limited by available light. In subsequent years the metalimnetic populations stratified at shallower depths, and as a result, the irradiance that reached the metalimnetic peak of biomass was 8.2% of the surface irradiance in 1981 and production in the 4- to 6-m layer was much higher.

The metalimnetic populations consisted of gas vacuolate cyanobacteria; buoyancy in these organisms is regulated such that the photosynthetic rate determined by light intensity does not exceed the rate at which inorganic nutrients can be assimilated for growth (Dinsdale and Walsby 1972). Stratification at shallower depths in 1981 can be interpreted in two ways. (1) Nutrient availability may have been greater, and this allowed the organisms to maintain neutral buoyancy at a higher light intensity. (2) Different species inhabited the metalimnion in 1981 (Table 3), including heterocystous species, which have a high energy requirement when fixing nitrogen gas (Zevenboom 1980). Perhaps stratification at shallower depths (and higher irradiance) was necessary for these species to meet higher energy demands. The appropriate physiological experiments have not been done to resolve these two hypotheses, although the threefold higher rainfall recorded in May 1981 and increased biomass found in the epilimnion during this year suggest that nutrient inputs may have been larger than in the previous years. If nutrient inputs are indeed increasing, the lake will contain dense phytoplankton populations in the epilimnion within a few years. New sources of nutrient loading are not obvious: the watershed of this lake includes some agricultural fields but is relatively small, and construction of homes along the shoreline has not increased during the past 3 yr.

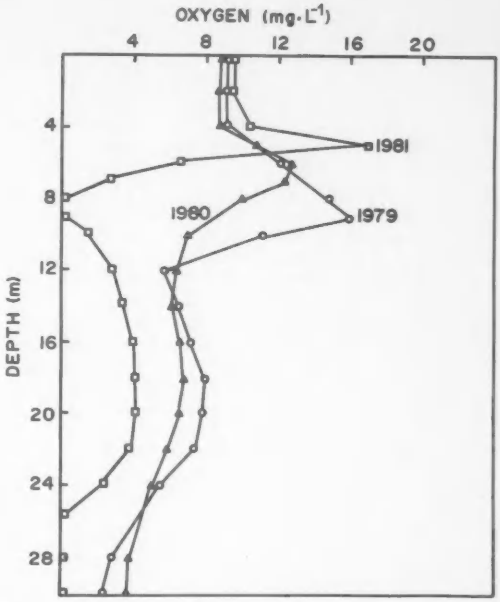

FIG. 6. Vertical distribution of dissolved oxygen in Crooked Lake during the 1st wk of July for 1979 (○), 1980 (△), and 1981 (□).

Stratification of the cyanobacteria at 5 m and increased primary production during 1981 affected other aspects of the lake. The vertical distribution of dissolved oxygen was different in 1981 (Fig. 6). Crooked Lake has been one of the few Indiana lakes to support a fish population of the planktivore *Coregonus artredii* (cisco). These fish inhabited the cold, oxygenated metalimnetic layers of the lake. However, because cyanobacteria stratified at a shallower depth in 1981, the oxygen accumulated in warmer water, and in the zone just below the cyanobacterial layer (a region in which respiration is relatively high) oxygen was depleted in early July. After this time, dead cisco were seen in the lake. The zooplankton density in the lake was threefold higher in 1981 than in previous years (K. M. Brown, Department of Biology, IUPU-Ft. Wayne, Ft. Wayne, IN, personal communication);

perhaps this was due in part to decreased predation by cisco. The lake bottom became anaerobic in early July 1981, about 6 wk earlier than in previous years. As a result, the sediments served as a source of phosphate in the hypolimnion for a longer period of time in 1981 (the dissolved inorganic phosphate concentration 1 m above the bottom sediment rose from 30 $\mu g\ P \cdot L^{-1}$ on June 29 to 300 $\mu g\ P \cdot L^{-1}$ on August 17) and might contribute to increased primary production in 1982. Crooked Lake will provide an interesting study of the interaction between factors that limit productivity in hardwater lakes, such as calcium carbonate precipitation (Wetzel 1972), and factors involved in eutrophication, such as the formation of anaerobic layers and subsequent release of phosphate from sediments.

In Crooked Lake, metalimnetic production was responsible for 30–45% of total production, a much larger proportion than was reported for Canadian lakes in the Experimental Lakes Area (Fee 1978). Simulations using the model demonstrated that, in general, metalimnetic production decreased as the light extinction coefficient increased, solar radiation decreased, or the depth at which the phytoplankton stratified increased. In most systems, the depth of stratification should be most important, because the species that form these deep layers (usually cyanobacteria or flagellates) can actively regulate their vertical position. Cyanobacteria have been shown to regulate their buoyancy in response to light intensity and nutrient concentration (Konopka 1982a). Thus, the quantitative importance of metalimnetic production in these lakes will be determined by the light intensity at which the phytoplankton can maintain their vertical position, as dictated by the interaction between nutrient and light availability at that stratum.

Acknowledgments

This research was supported by grants DEB-7904382 from the National Science Foundation and B-127:IND from the Office of Water Resources and Technology. I thank Lucky Bois and Tracy Miller for excellent technical assistance.

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Effect of Atmospheric Sulfur on the Composition of Three Adirondack Lakes

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GALLOWAY, J. N., C. L. SCHOFIELD, N. E. PETERS, G. R. HENDREY, AND E. R. ALTWICKER. 1983. Effect of atmospheric sulfur on the composition of three Adirondack lakes. Can. J. Fish. Aquat. Sci. 40: 799–806.

Three watershed–lake systems of the Integrated Lake–Watershed Acidification Study (ILWAS) were investigated to determine the effects of atmospheric deposition on the chemical compositions of oligotrophic lakes in the Adirondack Mountains of New York. Using the principles of watershed mass balance and electroneutrality of solutions, the following conclusions were drawn. (1) Annually, about 90% of the NH_4^+ and 50% of the NO_3^- from atmospheric deposition were retained in the systems. (2) In the Woods system, Cl^- was in steady state with respect to atmospheric deposition although both Panther and Sagamore systems had net losses, indicating watershed sources of Cl^-. (3) The losses of base cations from Panther and Sagamore were substantially greater than from the Woods system, reflecting the shallow soils of the latter. (4) The concentrations of SO_4^{2-} in the waters of the three systems were controlled by the atmospheric deposition of anthropogenic sulfur; in Woods and Panther, inputs (atmospheric deposition) equalled outputs (discharges from the lake outlets); in Sagamore, outputs exceeded inputs. (5) In 1978–80, concentrations of SO_4^{2-} were four to five times higher than historical values. These increased concentrations had caused either decreased alkalinities of surface waters or increased concentrations of base cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) or both. The former directly affects aquatic ecosystems; the latter directly affects terrestrial ecosystems because of increased rates of loss of the nutrients Ca, Mg, and K in the absence of resupply from primary weathering.

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Trois réseaux de bassins hydrographiques et de lacs de l'*Integrated Lake–Watershed Acidification Study* (ILWAS) ont été étudiés en vue de déterminer les effets des retombées atmosphériques sur la composition chimique de lacs oligotrophes des Adirondacks de l'État de New York. Utilisant les principes du bilan de masse des bassins et l'électroneutralité des solutions, on est arrivé aux conclusions suivantes : (1) sur base annuelle, environ 90 % de NH_4^+ et 50 % de NO_3^- des retombées atmosphériques sont retenus dans les réseaux; (2) dans le réseau Woods, Cl^- est en état d'équilibre par rapport à la retombée atmosphérique, bien que les réseaux Panther et Sagamore aient tous deux des pertes nettes, signe de la présence de Cl^- dans le bassin; (3) les pertes de cations basiques de Panther et Sagamore sont substantiellement plus fortes que celles du réseau Woods, conséquence des sols peu profonds de ce dernier; (4) les concentrations de SO_4^{2-} dans l'eau des trois réseaux sont réglées par la retombée atmosphérique de soufre anthropique; dans les réseaux Woods et Panther, les

Printed in Canada (J7015)
Imprimé au Canada (J7015)

entrées (retombées atmosphériques) égalent les sorties (débit des émissaires des lacs); dans le réseau Sagamore, les sorties dépassent les entrées; (5) en 1978−80, les concentrations de SO_4^{2-} étaient de quatre à cinq fois supérieures aux valeurs historiques. Comme résultat de ces concentrations accrues, il y eut soit diminution de l'alcalinité des eaux de surface, soit augmentation de concentration des cations basiques (Ca^{2+}, Mg^{2+}, Na^+, K^+) ou les deux. Le premier effet agit directement sur les écosystèmes aquatiques; le dernier directement sur les écosystèmes terrestres par suite de taux accrus de pertes des nutriments Ca, Mg et K dus à l'absence de renouvellement par décomposition primaire.

Received July 22, 1982
Accepted March 9, 1983

Reçu le 22 juillet 1982
Accepté le 9 mars 1983

THE alkalinities of oligotrophic lakes in the eastern United States range from about −100 to more than 1000 $\mu eq \cdot L^{-1}$.[1] The net result of alkalinity-producing (e.g. primary weathering) and acidity-producing (e.g. sulfide oxidation) processes determines the alkalinity of any lake.

Besides these natural processes, the rates of which control pH, an anthropogenic process — fossil-fuel combustion — acidifies sensitive aquatic ecosystems through acidic deposition (e.g. Galloway and Cowling 1978; Overrein et al. 1980; Hendrey et al. 1980). To understand the interaction of this additional process with the natural ones, in 1976 we selected three lakes that were receiving similar amounts of acidic deposition per unit area but that had daily alkalinities ranging from −100 to 300 $\mu eq \cdot L^{-1}$. This project, the Integrated Lake−Watershed Acidification Study (ILWAS), which began in 1977, was specifically designed to determine the dominant processes controlling the alkalinities of three oligotrophic lakes and why each lake responded differently to identical acidic deposition rates.

Apparently, the alkalinities of the three lake systems, which were hydrologically and chemically monitored, differ primarily because of different watershed morphologies and soil compositions (Newton and April 1982; Troutman and Peters 1982). The average monthly alkalinities of the lakes' surface waters were −10 $\mu eq \cdot L^{-1}$ for Woods, 30.6 $\mu eq \cdot L^{-1}$ for Sagamore, and 147 $\mu eq \cdot L^{-1}$ for Panther (Table 1). This paper addresses how these lakes and their associated watersheds (hereafter called "systems") responded to identical inputs of acidic deposition, why the responses differed, and what the effects of H_2SO_4 were on the systems.

Materials and Methods

LAKES STUDIED

Panther, Woods, and Sagamore lakes are in the Adirondack State Park, New York. The watersheds are forested with a mixture of hard and soft woods and have areas of 1.24, 2.07, and 49.7 km^2, respectively.

Flushing times are 264, 221, and 81.4 d and mean depths are 3.9, 3.5, and 10.5 m for Panther, Woods, and Sagamore lakes, respectively. Panther and Woods lakes are clear but Sagamore is brown, presumably from the humic and fulvic acids released from the significant bog development in the Sagamore watershed.

ELEMENTAL BUDGETS

To analyze the first year's data for ILWAS, Galloway et al. (1980a) compared total inputs (wet and dry atmospheric deposition) from annual hydrological and chemical data to the total outputs (stream discharge from a lake) and concluded the following. (1) Woods system: the atmospheric deposition of H^+ is sufficient to account for all the net loss of basic cations from the system. (2) Sagamore and Panther systems: the atmospheric deposition of H^+ is less than the net loss of basic cations; therefore, because base cation production is dependent on H^+ interactions with mineral surfaces, the net internal generation of H^+ for Sagamore and Panther is at least of similar magnitude to the deposition of H^+ from the atmosphere.

To explain the different abilities of the three systems to neutralize atmospheric acids, Galloway et al. (1980a) developed the following hypothesis:

> How long atmospheric H^+ remains in the soil and till of a system determines how that system will respond. On an annual basis, the Woods system, with a steeper topography and less soil than the Sagamore and Panther systems, will not be able to neutralize acidic deposition completely; however, Sagamore and Panther, with deeper soil and more till, will neutralize acidic deposition to greater degrees.

This hypothesis was developed from the analyses of input and output data covering only 1 yr. Although the use of annual mass balances has several advantages, a major disadvantage is the assumption that for conservative materials inputs balance outputs over 1 yr. This assumption may be incorrect because some materials may stay in the systems longer than 1 yr. To resolve this problem, we calculated the net flux for this study as a running sum and defined the function running net flux, RNF, as

$$\mathrm{RNF} = \sum_{i=1}^{n} (I - O)_i$$

where i is the time in months of observation, n is the number of months, I is the atmospheric deposition (input) of the element (equivalents per hectare), and O is the stream discharge (output) of the element from the lake outlet (equivalents per hectare). This function is best used graphically where RNF is plotted against time. Any point on the graph,

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[1] $\mu eq \cdot L^{-1}$ (microequivalents per litre) are used here because the principle of electroneutrality of solutions is used in discussing the data; $\mu eq \cdot L^{-1}$ may be converted to $\mu mol/L$ by dividing the ion concentration by its electrical charge.

TABLE 1. Average annual concentrations ($\mu eq \cdot L^{-1}$) in the water leaving the Woods, Panther, and Sagamore systems calculated by averaging weekly samples from the lake outlets over a 3-yr period (1 Jan. 1978 to 31 Dec. 1980).

	Woods	Sagamore	Panther
pH	4.7	5.6	6.2
Alkalinity	−10.0	30.6	147
SO_4	126	163	123
NO_3	19.4	24.1	23.4
Cl	9.10	16.6	12.6
H	18.2	2.3	0.69
NH_4	3.16	0.68	1.64
Ca	73.1	147	207
Mg	18.6	55	52
Na	19.3	35	41
K	6.2	12	12
Al	13.65	1.51	0.50

TABLE 2. Estimated concentrations and deposition velocities (V_d) of SO_2 in summer and winter.

	SO_2 ($\mu g\ S \cdot m^{-3}$)	V_d ($cm \cdot s^{-1}$)	Deposition ($eq \cdot ha^{-1} \cdot mo^{-1}$)
Summer (June–Oct.)	2	0.5	15
Winter (Nov.–May)	4	0.3	20

$t = i$, represents the net flux between that time and $t = 0$, the start of the project (1 Jan. 1978). The RNF (1) identifies the elements in a steady state (inputs = outputs), (2) estimates the rate of loss or gain of an element not in a steady state in internal reservoirs (soil, vegetation, etc.), and (3) identifies the critical periods of ion uptake or release of an element.

If RNF = 0, a system is (at that point in time) in a steady state (inputs = outputs) relative to $t = 0$. If RNF > 0, a system had a net gain of the material; a small increase in the slope of the RNF represents an increase in the storage rate of an internal reservoir. If RNF < 0, a system has a net loss of the material; a small decrease in slope represents an increase in the rate of loss from an internal reservoir. A disadvantage of the RNF is that, as time progresses, the changes in slope become less sensitive indicators of the seasonal changes in flux.

To relate the RNF, a difference function, to the total input flux so that we could determine the relative degree of loss or gain of each system, we calculated a retention index, RI:

$$RI = \frac{RNF}{\sum_{i=1}^{n} (\text{inputs})_i}.$$

The values of RI ranged from 1 to $-\infty$. If RI = 1, a system retained all the material deposited from the atmosphere (i.e. $\sum_i O_i = 0$). If RI = 0, a system was in a perfect steady state (i.e. it lost the same amount of the material as it received from the atmosphere: $\sum_i I_i = \sum_i O_i$). If RI = −1, a system lost twice as much material as it received from the atmosphere. If RI = −2 (−3, −4, etc.), a system lost three times (four times, five times, etc.) as much material as it initially received from the atmosphere.

DATA

We used four types of data to calculate the RNFs and RIs.

1) Atmospheric deposition (1 Jan. to 31 Mar. 1978): bulk precipitation (rain, snow, and dry fallout) was collected near Panther Lake by researchers from Cornell University and the University of Virginia using a bulk-precipitation collector (Likens et al. 1977). The samples were analyzed for pH (using a pH meter), SO_4^{2-}, NO_3^-, Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, and NH_4^+ at the University of Virginia using standard techniques on an atomic absorption spectrophotometer and a Technicon auto analyzer.

2) Atmospheric deposition (1 Apr. 1978 to 31 May 1980): wet deposition (rain and snow) and dry fallout were collected separately at four to seven sites in or near the three systems by researchers from Rensselaer Polytechnic Institute. Details of the sampling and analytical techniques were documented by Johannes and Altwicker (1980).

3) Dry deposition of SO_2 (1 Jan. 1978 to 31 May 1980): dry deposition of SO_2 was calculated as a function of season by estimating the concentration of SO_2 and its deposition velocity, V_d (Table 2). The estimated V_d allowed for variations with the time of day and the extent and type of forest canopy (see Sheih et al. 1979). The estimated concentrations of SO_2 were based on data available from the northeastern United States (Galloway and Whelpdale 1980) and measurements made in the ILWAS systems by researchers from Rensselaer Polytechnic Institute. Because of uncertainties about the magnitude of the deposition velocity and temporal variability of the atmospheric concentrations, the errors in the calculations of dry deposition were about ±75% (Galloway and Whelpdale 1980). Because dry deposition was about 30% of the total deposition, the error in calculating total atmospheric deposition was about ±20%. The ±20% error and a 10% error in calculating the wet deposition of sulfur (wet deposition was 70% of total deposition) made the total error in the calculation of atmospheric deposition of sulfur about ±30%.

4) Discharge of elements from lake outlets (1 Jan. 1978 to 31 May 1980): discharge data used in the load calculations were determined from continuous stage measurements at gauged sites at the lake outlets and the relationships between discharge and state measurements established using methods of the USGS, Water Resources Division (Corbett 1962).

Stream samples were taken in acid-washed polypropylene bottles at the hydrologic gauging stations at least weekly, except in the winter of 1977–78 when samples were taken biweekly. Lake samples were taken at six depths either weekly or every other week using peristaltic sampling pumps.

Conductivity and pH were measured the same day the samples were taken. Alkalinity was determined by titration (Gran 1950) at a field laboratory within 1–2 wk of the sample collection. The samples were then mailed to the University of Virginia where Ca^{2+}, Na^+, K^+, and Mg^{2+} were measured with an Instrumentation Laboratory 751 atomic absorption spectrophotometer; SO_4^{2-}, NO_3^-, Cl^-, NH_4^+, and H_4SiO_4 were measured using automated colorimetric techniques (Galloway et al. 1982). The samples were kept at 4°C until

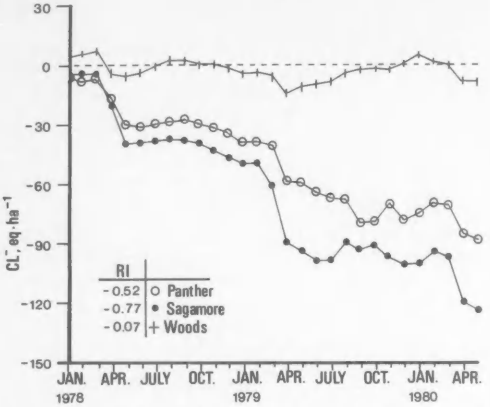

FIG. 1. The RNF and RI for Cl in the Woods, Sagamore, and Panther systems. The magnitude of the error is ±15% based on measurements of accuracy and precision of the chemical and hydrological data.

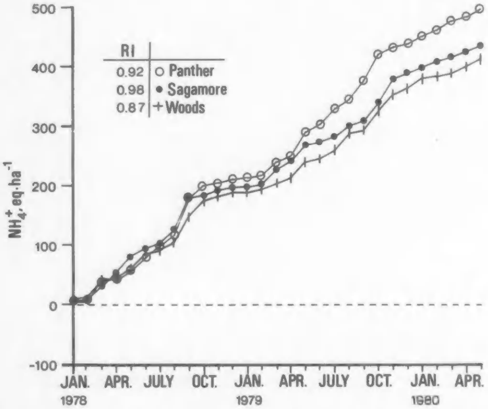

FIG. 2. The RNF and RI for NH_4 in the Woods, Sagamore, and Panther systems (see legend to Fig. 1 for error estimate).

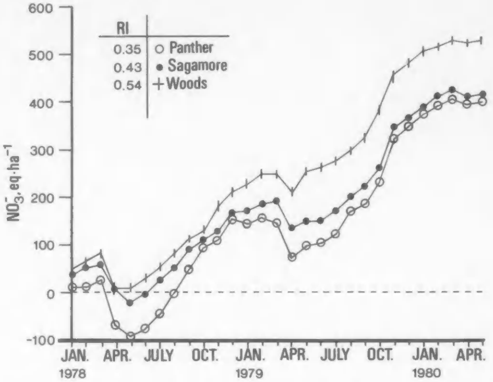

FIG. 3. The RNF and RI for NO_3 in the Woods, Sagamore, and Panther systems (see legend to Fig. 1 for error estimate).

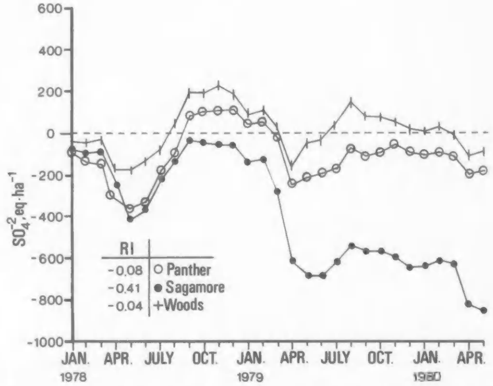

FIG. 4. The RNF and RI for SO_4 in the Woods, Sagamore, and Panther systems (see legend to Fig. 1 for error estimate).

analyzed, which was within 4 wk.

Tests of sample stabilities indicated that only NH_4^+ concentrations changed significantly between collection and analysis. Because NH_4^+ concentrations were low, they had little effect on the chemical integrity of the samples or the anion−cation balance.

Results and Discussions

CHLORIDE

Chloride is commonly used as an atmospheric tracer in watershed studies because of its low concentration in rocks and soils and its low reactivity, assuming there are no internal sources of sinks.

Of the three ILWAS systems, only Woods had no apparent internal source of Cl^- (Fig. 1). The RNF for the Woods system fluctuated seasonally around zero with an RI of −0.07, which is essentially equal to zero, given the errors involved in measuring elemental fluxes. These data are similar to those reported by Overrein et al. (1980) for a lake−watershed system in Norway. However, the net losses of Cl^- from the Panther and Sagamore systems (RI = −0.52 and −0.77, respectively) implied sources within the systems. This raised two questions: what were the sources? and why was the Woods system unaffected?

Of the primary minerals in the bedrock and surface materials (see Troutman and Peters 1982), hornblende is the only potential source of Cl^- (R. April, Colgate College, Hamilton, NY, personal communication). Buddington and Leonard (1953) found that Cl^- concentrations averaged about 0.6% by weight in hornblendes from the Adirondacks. April and Newton (1983) analyzed hornblendes from the ILWAS

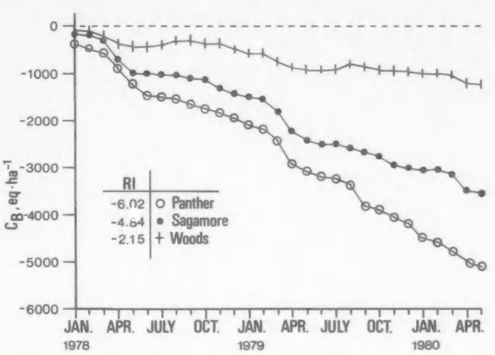

FIG. 5. The RNF and RI for C_B (Ca^{2+}, Mg^{2+}, Na^+, K^+) in the Woods, Sagamore, and Panther systems (see legend to Fig. 1 for error estimate).

systems and found average Cl^- concentrations that corroborate the data from Buddington and Leonard. These relatively large concentrations were probably adequate to have accounted for the internal sources of Cl^- within the Panther and Sagamore systems. The soil and surficial materials in Woods and Panther contain similar percentages of hornblende (Troutman and Peters 1982). However, in Woods, the surficial material in the basin is thin and discontinuous (Troutman and Peters 1982) and the soil is less permeable than in Panther (Newton and April 1982). Therefore, the acidic atmospheric water resided with the neutralizing mineral horizons in the Woods system for less time than in the Panther system; hence, the Cl^- values of the Woods system were lower than those of the Panther system.

AMMONIUM

Each ILWAS system gained NH_4^+ (Fig. 2). Based on the RIs, the Woods, Sagamore, and Panther systems retained 87, 98, and 92%, respectively, of NH_4^+ deposited from the atmosphere. These retentions occurred primarily in the spring and summer months when NH_4^+ is used by forested ecosystems (Likens et al. 1977).

NITRATE

The net retention of NO_3^- (Fig. 3) was expected because forested ecosystems are probably nitrogen-limited. However, strong seasonal fluctuations in the RNFs were also observed; these fluctuations and the difference in RIs between the Woods and Panther systems helped to determine how the three systems responded to identical inputs of atmospheric deposition.

The slopes of the NO_3^- RNFs changed directions during different seasons. In the summer, when productivities were highest, the systems rapidly assimilated NO_3^-. In the winter, because of the dormancies of the biological components, the systems were in approximately steady state (i.e. slopes of RNF ~ 0). In the spring, NO_3^- was rapidly released from the systems from the melting of the snowpack (Galloway et al. 1980b).

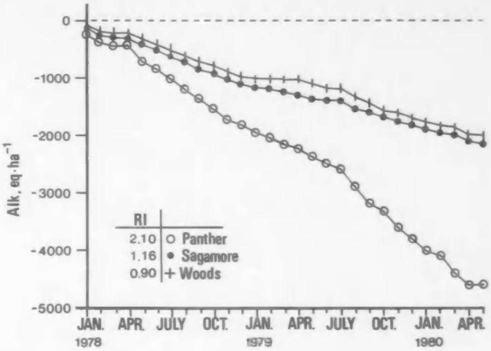

FIG. 6. The RNF and RI for alkalinity in the Woods, Sagamore, and Panther systems (see legend to Fig. 1 for error estimate).

More NO_3^- was accumulating in the Woods system than in Panther or Sagamore because either (1) Panther and Sagamore had larger internal sources of NO_3^- (i.e. nitrification) than did Woods or (2) Woods had a larger net accrual of biomass than the other two systems. We do not yet know which of the two possibilities is correct.

The RIs for NO_3^- in the Woods, Sagamore, and Panther systems indicated that 54, 43, and 35%, respectively, of the NO_3^- deposited from the atmosphere was retained within each system, presumably in the biomass. These percentages, however, were maximum estimates because of the unmeasured dry deposition of gaseous nitrogen compounds (e.g. HNO_3, NH_3, NO, NO_2).

SULFATE

The distinct seasonal variations in the SO_4^{2-} fluxes oscillated around zero for Woods and Panther (Fig. 4). The RIs indicated that Panther and Woods were in steady state, i.e. neither system was gaining or losing sulfur. However, the Sagamore system had a net loss of SO_4^{2-}. Because the three systems received essentially identical atmospheric loadings per unit area, there was an internal source of sulfur within Sagamore.

The mean SO_4^{2-} concentration in excess of that supplied from seasalt in Sagamore outlet was ~160 μeq·L^{-1}; but in Panther and Woods it was only ~120 μeq·L^{-1}, implying an internal source of SO_4^{2-} for Sagamore (Church and Galloway 1983). Also, the surface water of Sagamore Lake was brown, presumably from the release of humic and fulvic acids from bogs that were not present to any appreciable extent in the other two systems. The SO_4^{2-} concentration in the stream below the bogs was higher than above them (J. N. Galloway, unpublished data), which suggests that reduced sulfur was oxidized to SO_4^{2-} in the bogs.

BASE CATIONS (C_B)

All three systems lost C_B (Fig. 5). The following rates

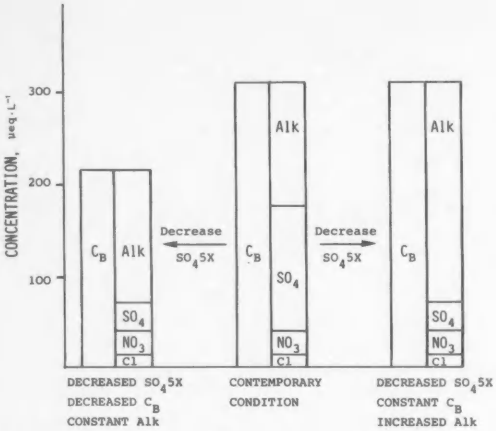

FIG. 7. The effect a fivefold reduction in SO_4 concentration would have on the surface-water composition of Panther Lake. The center bar graph was based on the concentration at the time of our research (data from Table 1).

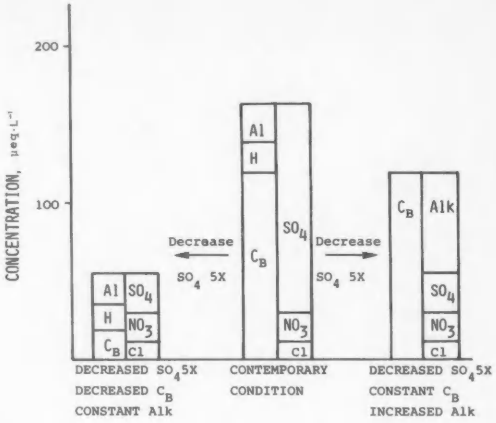

FIG. 8. The effect a fivefold reduction in SO_4 concentration would have on the surface-water composition of Woods Lake. The center bar graph was based on the concentration at the time of our research (data from Table 1).

of net losses were calculated from data spanning 2 yr (1 Jan. 1978 to 31 Dec. 1979): Woods, 496 eq·ha^{-1}·yr^{-1}; Sagamore, 1498 eq·ha^{-1}·yr^{-1}; Panther, 2182 eq·ha^{-1}·yr^{-1}.

The predominant minerals found in these watersheds — sodic plagioclase, potassium feldspar, and hornblende (Troutman and Peters 1982) — weather through hydrogen ion transfer. The overall reaction proceeds irreversibly as follows:

$$\text{K-feldspar} + \text{plagioclase} + \text{hornblende} + H^+ \rightarrow C_B + \text{clay minerals (vermiculite)} + \text{metal oxides} + \text{metal hydroxides} + H_4SiO_4.$$

C_B is also lost by ion-exchange reactions in which a proton may exchange for C_B on an ion-exchange site.

The questions that needed to be addressed were (1) why did the three systems have different losses of C_B? and (2) what was the influence of acidic deposition on the production of C_B? The three systems exported different amounts of C_B for essentially the same reasons they exported different amounts of Cl^-. In the Woods system, because the atmospheric water had less contact with less soil and till than in the Sagamore and Panther systems (Newton and April 1982; Troutman and Peters 1982), less C_B was produced. Panther exported more C_B than Sagamore. This was contrary to the behavior of Cl^- because there was more Cl^--bearing hornblende in Sagamore than in Panther. The second question will be addressed in the discussion below of the interactions between C_B, Alk, and SO_4^{2-}.

ALKALINITY

All three systems had net losses of alkalinity (Alk) (Fig. 6). RNFs and RIs for Woods and Sagamore were similar because the alkalinities of their stream discharges were very near zero (−10 and +31 µeq·L^{-1}, respectively). Therefore, the RNFs for the alkalinities of these two systems were controlled by the inputs of atmospheric deposition, which were virtually identical. Panther, which had a much higher alkalinity in its surface water (~147 µeq·L^{-1}), had a greater net loss of Alk. The longer contact time of acidic atmospheric deposition with the neutralizing minerals in Panther than in Woods could explain the larger Alk as corroborated by the net chloride released.

INFLUENCE OF ATMOSPHERIC DEPOSITION ON ELEMENT CYCLING

To determine how acidic deposition had affected the ILWAS waters, the sources of the major components of those waters (Alk, C_B, SO_4^{2-}) and the interactions of these components had to be determined.

The Alk and C_B in the ILWAS waters resulted from biogeochemical processes within the systems — specifically, the fundamental weathering reaction occurring when CO_2 from respiration in the presence of H_2O reacts with bedrock and soils to produce Alk and C_B. Once weathering had produced Alk and C_B, internal processes (e.g. vegetation growth, ion-exchange reactions) could have caused the levels of Alk and C_B to fluctuate.

The possible sources of SO_4^{2-} were the atmospheric deposition and the internal oxidation of organic sulfur and sulfide minerals. We concluded that the dominant source of SO_4^{2-} in each ILWAS system was atmospheric deposition because (1) sulfide minerals were virtually absent from each system (Troutman and Peters 1982), (2) reduced sulfur was rarely found in the watersheds of any system (except in Sagamore as previously noted), and (3) the RNFs for SO_4^{2-} indicated that each system (except Sagamore) was in steady state with sulfur relative to atmospheric inputs.

Because over 90% of the atmospheric sulfur in eastern North America is from fossil-fuel combustion and the smelting of sulfide ores (Galloway and Whelpdale 1980; Yuen et al. 1979), we considered how decreases in sulfur emissions to the atmosphere might affect the chemical composition of the ILWAS streams and lakes.

We knew that (1) Woods and Panther were in steady state with respect to sulfur, (2) more than 90% of the sulfur in the atmosphere of eastern North America is anthropogenic, and (3) the major anions in the ILWAS systems were SO_4^{2-} and HCO_3^- (except in Woods where they were SO_4^{2-} and NO_3^-). Therefore, we assumed that if the anthropogenic sulfur sources to the atmosphere were turned off, then sulfur depositions (and the corresponding acidity) from the atmosphere to the ILWAS systems would decrease by about a factor of five (~80%),[2] which would cause corresponding decreases in sulfur discharges from the Woods and Panther systems. If SO_4^{2-} concentrations were to decrease and the systems remain in steady state with respect to sulfur, then one of two extremes could happen to conserve the electroneutrality of the water. Either (1) C_B would decrease, which would mean that atmospheric SO_4^{2-} and the associated H^+ were promoting strong acid weathering or stripping C_B from the cation-adsorption sites or (2) alkalinity would increase, which would mean that the H^+ was neutralizing HCO_3^- and that there was no linkage between C_B output and SO_4^{2-} deposition. The exact magnitudes of these effects are illustrated in Fig. 7 and 8. It is important to realize that these two extremes represented boundary conditions. In reality, the actual responses of the systems to decreased SO_4^{2-} and H^+ deposition would be somewhere between the extremes. In addition, there would not be an immediate response of aquatic systems to the reduction of atmospheric deposition of sulfur. Depending on the characteristics of the terrestrial and aquatic systems it might be years or decades before a new equilibrium was reached.

For the Panther system (Fig. 7), we imposed an 80% decrease in SO_4^{2-} concentration on the lake based on data from Table 1. The charge budget on the right in Fig. 7 shows that the loss of SO_4^{2-} would be compensated by an increase in Alk so that the equilibrium pH of Panther associated with the reduced SO_4^{2-} deposition would be about 6.4 compared with the actual value of 6.2. The budget to the left shows that the loss of SO_4^{2-} would be compensated by a decrease in C_B because of less weathering loss of C_B by atmospheric H^+. This loss would be a 32% reduction (312−213 $\mu eq \cdot L^{-1}$ of C_B) in the concentration of C_B in Panther Lake outlet and would correspond to a change in C_B from 2182 to 1483 $eq \cdot ha^{-1} \cdot yr^{-1}$. Therefore, if the atmospheric deposition of sulfur were reduced by a factor of five, the maximum effects on the Panther system would be either a 67% increase in lake alkalinity or a 36% decrease in the loss of C_B (Ca^{2+}, Mg^{2+}, Na^+, and K^+) from the watershed. The actual effect would probably be some point between the two extremes, representing a partial response from both possibilities. Current research is attempting to locate this point.

Two extremes could also take place in the chemistry of Woods Lake if the SO_4^{2-} concentration were reduced by a factor of five. If Alk were increased (Fig. 8, right-hand column), the change would be from −18 to +64 $\mu eq \cdot L^{-1}$ — an increase that would result in a pH of about 5.8 compared with the actual mean pH of 4.7. This calculation of an estimated future pH was based on the assumption that the current concentration of CO_2 in the water would remain the same and that the carbonate species were the only ones that contributed to alkalinity. The first assumption was untestable and served only as a basis for comparison. The second assumption was true for Panther because the high pH precluded the effects of other buffering systems (e.g. Al and organic compounds). However, for the Woods system, the second assumption was less appropriate because of the low pH and the buffering by Al and organic compounds. The estimate of 5.8 did, however, provide an upper limit to the pH.

The other extreme in Woods Lake would be if the reduction in the SO_4^{2-} were balanced by a reduction in C_B. Because SO_4^{2-} and C_B dominated the solution chemistry in Woods Lake when we studied the system, an 80% decrease in SO_4^{2-} would produce an 87% decrease in C_B concentration (117−16 $\mu eq/L$), resulting in a decrease in the net flux of C_B from 496 to 64 $eq \cdot ha^{-1} \cdot yr^{-1}$. The relationship between SO_4^{2-} and C_B is more complex than this simple charge balance. Ultimately, it comes down to the effect of H^+ on C_B production. Again, the exact effect of reduced SO_4^{2-} concentration would probably be represented by a point between these two extremes. Since the alkalinity of the lake was approximately zero, the lake would probably attain a mean alkalinity greater than zero.

Conclusions

The analyses of 29 mo of data on the fluxes of SO_4^{2-}, NO_3^-, C_B, NH_4^+, Alk, and Cl^- into and out of three watershed lake systems in the Adirondack Mountains showed that the systems assimilated most of the NH_4^+ from atmospheric deposition and about half of the NO_3^-. The analyses of the net loss of C_B from the three systems supported an earlier hypothesis (Galloway et al. 1980a) that the relative loss of C_B is controlled by the depth of the soil in the three systems.

The atmosphere was the major source of sulfur in the streams and lakes of the three systems. A 75−80% decrease in the levels of atmospheric sulfate could cause an increase in alkalinity or a decrease in the sum of the C_B in surface waters from the lake outlets of two headwater basins from the Adirondack Mountains of New York State. Although the actual effect would be a combination of these two results, the alkalinity of the more acidic of the two systems would most probably become positive.

Woods and Panther lakes spanned an approximate range of lakes susceptible to acidification by acidic deposition; their responses to changes in sulfur deposition can be used to estimate the responses of many lakes in the northeastern United States and southeastern Canada. Specifically, for systems similar to the Woods system (i.e. waters with low alkalinity and in a steady state with respect to sulfur), reductions in sulfur deposition would cause increases in alkalinity and reductions in the loss of C_B from the terrestrial component of the system.

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[2] This would only occur if the atmosphere acted as a passive reservoir where inputs equalled outputs. Because the oxidation of SO_2 may be a nonlinear process, this may not be true (Altwicker et al. 1981). However, for the sake of discussion, we will assume linearity and that an 80% reduction in sulfur inputs to the atmosphere would result in an equal reduction in deposition.

Acknowledgments

This study was primarily funded by the Electric Power Research Institute. We thank the Adirondack League Club, the Sagamore Conference Center, Ranger William Marleau, and the International Paper Company for permission to work on these lakes and their enthusiastic support of the project. Also, a special thanks to Brenda Morris for her help and understanding during all phases of the ILWAS research. We also thank Mary-Scott Marston for her help in preparing this manuscript.

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NOTES

Measurement of Primary Productivity in Dense Algal Cultures

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GENTNER, S-R. 1983. Measurement of primary productivity in dense algal cultures. Can. J. Fish. Aquat. Sci. 40: 807–810.

The ^{14}C-bicarbonate method of primary productivity measurement was modified for dense algal cultures by the addition of $NaHCO_3$ to avoid exhaustion of carbon source. Using this method plus a modified procedure for scintillation counting of limited sample size, six species of chlorophyean algae were studied daily throughout the growth cycle. *Scenedesmus acutus* was the most productive with a peak productivity of 5.3 mg $C \cdot L^{-1} \cdot h^{-1}$ at 9.7×10^3 lx during late exponential phase. Peak productivity values (mg $C \cdot L^{-1} \cdot h^{-1}$) for the other species were 2 for *Chlorella vulgaris*, 2.3 for *Ankistrodesmus braunii*, and 3.4, 3.5, and 3.6 mg $C \cdot L^{-1} \cdot h^{-1}$ for *Scenedesmus basiliensis*, *S. bijugatus*, and *S. parisiensis*, respectively.

GENTNER, S-R. 1983. Measurement of primary productivity in dense algal cultures. Can. J. Fish. Aquat. Sci. 40: 807–810.

La méthode de mesure de la productivité primaire à l'aide de ^{14}C-bicarbonate a été modifiée, dans le cas de cultures algales denses, par addition de $NaHCO_3$, afin d'éviter l'épuisement de la source de carbone. Cette méthode, couplée à une technique modifiée de comptage par scintillation d'échantillons de taille limitée, a permis de suivre quotidiennement six espèces de chlorophycés durant le cycle de croissance complet. *Scenedesmus acutus* est l'espèce la plus productive, avec productivité maximale de 5,3 mg $C \cdot L^{-1} \cdot h^{-1}$ à $9{,}7 \times 10^3$ lx à la fin de la phase exponentielle. Les pics de productivité (mg $C \cdot L^{-1} \cdot h^{-1}$) des autres espèces sont : 2 pour *Chlorella vulgaris*, 2,3 pour *Ankistrodesmus braunii*, et 3,4, 3,5 et 3,6 pour *Scenedesmus basiliensis*, *S. bijugatus* et *S. parisiensis* respectivement.

Received July 26, 1982
Accepted February 22, 1983

Reçu le 26 juillet 1982
Accepté le 22 février 1983

THE use of ^{14}C-carbonate (Steemann Nielson 1952; Vollenweider 1969) and ^{14}C-bicarbonate (Berman and Pollingher 1974; Plumb and Lee 1979) for measurement of algal primary productivity is well established for naturally occurring (i.e. nondense) populations. Productivity is defined as the rate of carbon assimilation per unit volume (mg $C \cdot L^{-1} \cdot h^{-1}$). Schindler and Fee (1973) drew attention to the inaccuracy possible under limiting carbon conditions in closed situations whereby primary productivity is underestimated. However, this note shows that with certain modifications the ^{14}C method can be used to study productivity at all stages of growth (i.e. all densities), and thus the peak productivities of most algal species can be compared.

Materials and methods — Six species of physiologically similar algae were grown under identical conditions: *Ankistrodesmus braunii* (Naeg.) Collins, *Chlorella vulgaris* Beijerinck, *Scenedesmus acutus* Meyen f. *alternans* (Utex 72), *S. basiliensis* Vischer (Utex 79), *S. bijugatus* var. *seriatus* Chodat, and *S. parisiensis* (Utex 1583); all are available from the Culture Collection, University of Texas. Algal strains were cultured at 20°C in 4 L of Krauss' (1953) medium with a 12-h photoperiod (9.7×10^3 lx); the inoculum was chosen to give an initial concentration of $\sim 2 \times 10^4$ cells$\cdot$mL^{-1}. Cultures were stirred and bubbled with 5% CO_2 in air. Once each culture reached early log phase, daily measurements of cell number, dry weight, and primary productivity were taken; measurements extended at least 3 d into stationary phase.

To measure primary productivity cells were centrifuged and resuspended in filtered (0.22 μm Millipore HA) 20°C water from Maskinonge Lake, an oligotrophic lake on the property of Chalk River Nuclear Laboratories. Maskinonge Lake water was used only for convenience, since the results of short-term productivity experiments in Maskinonge Lake water and Krauss' medium were indistinguishable. Cells

Printed in Canada (J7021)
Imprimé au Canada (J7021)

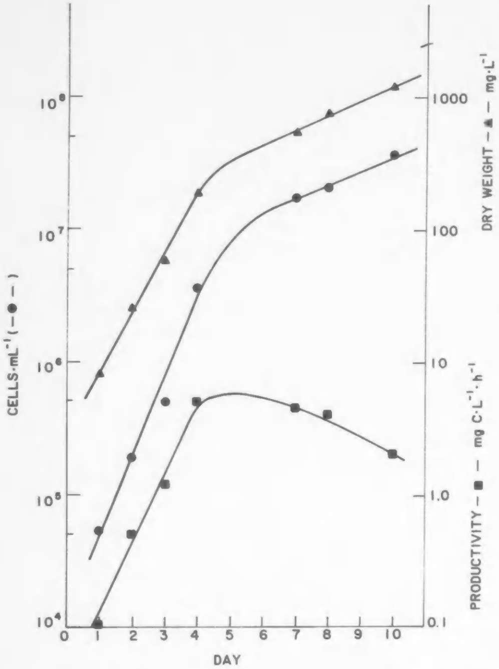


FIG. 1. Cells·mL^{-1} (-●-), dry weight in mg·L^{-1} (-▲-) and primary productivity (-■-) of *Scenedesmus acutus* as a function of growth time.

were incubated with an incident light of 9.7 × 10^3 lx and ^{14}C-labeled $NaHCO_3$ at 185 kBq·L^{-1} for 4 h. Unlabeled bicarbonate (usually 500 μg $NaHCO_3$·mL^{-1} or less; max. 800 μg·mL^{-1}) was added to the assays to assure that non-availability of bicarbonate was not limiting uptake.

For each measurement, duplicate samples were filtered onto Gelman 0.45 μm Metricel® filters and rinsed with distilled water using approximately twice the sample volume. Each filter, carrying retained cells, was dissolved in 10 mL scintillator (dioxane-based), and the ^{14}C content was determined in a Searle Mark III scintillation counter using the sample channels ratio (SCR) to correct for loss of counting efficiency. Color quenching was avoided by filtering a maximum permissible number of cells determined on the basis of culture age and species.

Results and discussion — The addition of $NaHCO_3$ to dense experimental cultures was necessitated by the depletion of all available carbon (5 ± 0.75 μg·mL^{-1}) by even mid-logarithmic phase cultures in the 4-h fixation period. To be sure that productivity was unchanged by the addition of bicarbonate, increasing amounts of $NaHCO_3$ up to 2600 μg·mL^{-1} were added to cultures of *Scenedesmus basiliensis* at 2 × 10^5 cells·mL^{-1}; there was no effect on carbon assimilation. For all species at higher cell densities, additions >600 μg $NaHCO_3$·mL^{-1} either did not change the fixation rate or increased it at most 15%. Schindler et al. (1980), in contrast, found a slight decrease in productivity due to a pH shift when carbon was added to short-term bottle assays. Thomas (1964) diluted dense cultures for experiments by a factor of 5, presumably to avoid exhausting the carbon source. However, increased light availability will have changed the productivity from that of denser cultures. If the system will tolerate it, addition of bicarbonate seems to be the only means to measure accurately carbon assimilation using ^{14}C.

It was found essential to limit the amount of culture filtered for scintillation counting to avoid color quenching. Pugh (1973) bleached color-quenched samples for 24 h under strong light. He also observed that SCR fails to correct for samples ≥1 mg dry weight. Bleaching can be avoided if the limits for color quenching are found for each algal species. For instance, in the case of *S. basiliensis*, 1 × 10^6 cells can always be counted without color quenching occurring. Avoiding the problem of color quenching also avoids the weight limitation on samples: 1 × 10^6 cells of *S. basiliensis* weighs <60 μg.

As is known, primary productivity of a culture increases as cell numbers increase during exponential growth, reaches a maximum during late exponential/early stationary phase, and thereafter falls as the culture goes further into stationary phase (Fig. 1). Productivity of different species is most readily compared on plots of productivity vs. culture density (Fig. 2a–f). These figures illustrate the zones of maximum productivity of culture. If, however, carbon assimilation per cell is considered, the greatest productivity occurs in cultures with concentrations of less than 1.5–2.5 × 10^6 cells·mL^{-1}, because carbon assimilation per cell falls after mid-logarithmic phase. However, on a per unit volume basis, the greater cell numbers at late exponential/early stationary phase outweigh this factor and the culture as a whole is at its most productive.

Scenedesmus acutus (Fig. 2c), with a peak productivity of 5.3 mg C·L^{-1}·h^{-1}, exhibits over twice the maximum productivity of *Ankistrodesmus* or *Chorella* and shows higher productivity than the other *Scenedesmus* species. In fact, a broad portion of its curve (range 3 × 10^6 – 2 × 10^7 cells·mL^{-1}) shows productivity >4 mg C·L^{-1}·h^{-1}, a value not attained by the other algae. The larger size of the *S. acutus* cells undoubtedly accounts for its higher productivity, all other characteristics of growth among these species being approximately equal. Since the dry weight·mL^{-1} of *S. acutus* cultures is high also, during peak productivity only about 2.1 L of culture would be needed to produce 1 g dry weight. From this comparison *S. acutus* could be selected as the most productive on the basis of smallest volume per gram dry weight coupled with the highest productivity.

FIG. 3. Productivity curves for *S. acutus* (a) and *S. parisiensis* (b) plotted as a function of cells·mL^{-1}. Results from two experiments where light intensity was 19.4 × 10^3 lx (-○-△-) are compared to results when light intensity was 9.7 × 10^3 lx (-●-▲-, as in Fig. 2c and f).

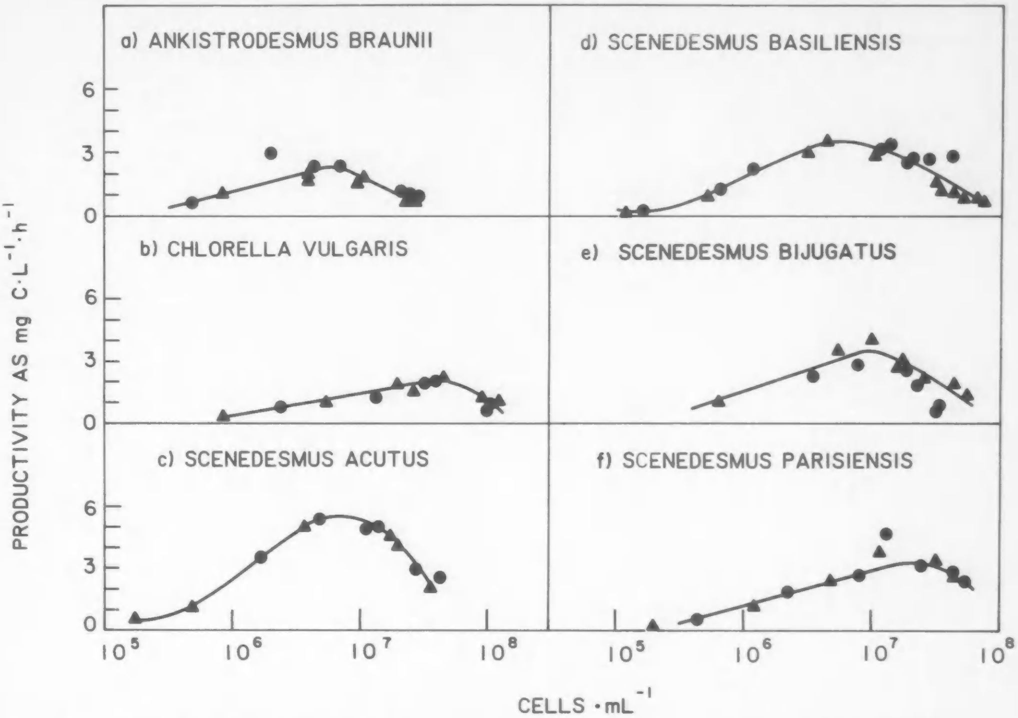
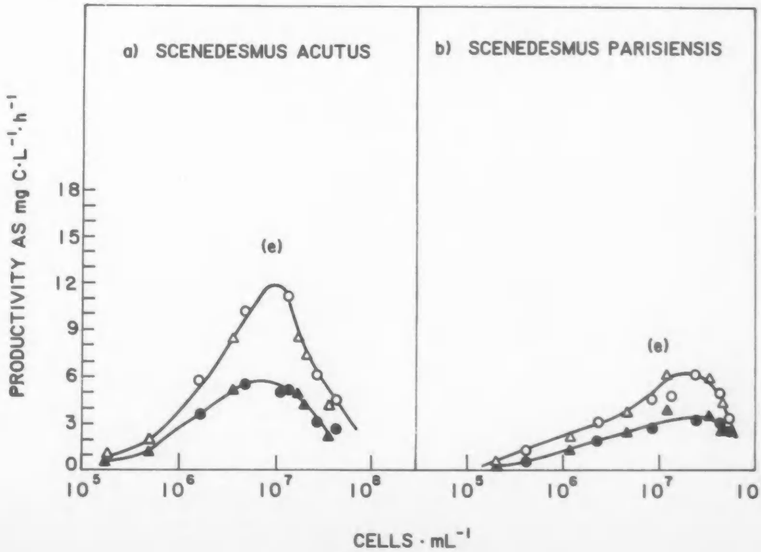


FIG. 2 (a–f). Productivities of the six species of algae plotted as a function of cells·mL^{-1}. Cells·mL^{-1} was used to establish the sampling place for maximum productivity in the culture. Two separate experiments are shown in each case: -●- and -▲- distinguish the results obtained.



At high cell densities, light shielding within the culture may limit productivity. As shown in Fig. 3a and b, doubling the light intensity causes only a small increase in productivity during early and mid-exponential phases but approximately doubles productivity in late exponential/early stationary phases. However, the cell density for maximum productivity is the same with either 9.7×10^3 lx or 19.4×10^3 lx, indicating that the drop in productivity observed as cells enter stationary phase is not due primarily to diminished light availability but rather reflects the physiological state of the cell.

The ^{14}C primary productivity method, modified by the addition of $NaHCO_3$ to dense experimental cultures, is therefore a feasible way to determine maximum productivity, as in aquaculture studies. For the six species studied, periods of peak productivity occur at late exponential/early stationary phase. Although the six species are remarkably similar for the parameters measured, small differences are easily detectable.

Acknowledgments — I wish to thank Dr N. E. Gentner for research suggestions and Mr J. M. Judd and Dr R. V. Osborne for their help in preparing the manuscript.

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Phosphorus Release by Three Kinds of Benthic Invertebrates: Effects of Substrate and Water Medium[1]

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NALEPA, T. F., W. S. GARDNER, AND J. M. MALCZYK. 1983. Phosphorus release by three kinds of benthic invertebrates: effects of substrate and water medium. Can. J. Fish. Aquat. Sci. 40: 810–813.

The effects of a sand substrate (presence vs. absence) and type of water medium (distilled or lake) on phosphorus excretion rates of tubificids, chironomids, and the amphipod *Pontoporeia hoyi* were determined. In contrast to previous studies on respiration rates, the presence or absence of a substrate did not significantly affect the excretion rates of any of the three taxa. Realistic determinations of P excretion can thus be obtained without a substrate present; this simplifies the approach to such determinations. Excretion rates of tubificids and chironomids were not affected by the type of medium, but the excretion rate of *P. hoyi* was slightly (but significantly) higher in distilled water than in lake water.

NALEPA, T. F., W. S. GARDNER, AND J. M. MALCZYK. 1983. Phosphorus release by three kinds of benthic invertebrates: effects of substrate and water medium. Can. J. Fish. Aquat. Sci. 40: 810–813.

On a mesuré les effets d'un substrat de sable (présence vs absence) et du type de milieu aqueux (eau distillée ou eau de lac) sur les taux d'excrétion de tubificides, de chironomides et de l'amphipode *Pontoporeia hoyi*. Contrairement à des études antérieures sur les taux de respiration, les taux d'excrétion de l'un ou l'autre des trois taxons ne sont pas influencés pour

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[1] GLERL Contribution No. 343.

Printed in Canada (J7134)
Imprimé au Canada (J7134)

la peine par la présence ou l'absence d'un substrat. On peut donc effectuer des mesures réalistes de l'excrétion de P en l'absence de substrat; de telles déterminations s'en trouvent simplifiées d'autant. Les taux d'excrétion des tubificides et des chironomides ne sont pas affectés par le type de milieu, tandis que celui de *P. hoyi* est légèrement (mais significativement) plus élevé dans de l'eau distillée que dans de l'eau de lac.

Received November 26, 1982
Accepted February 25, 1983

Reçu le 26 novembre 1982
Accepté le 25 février 1983

ACCURATE estimates of nutrient excretion by benthic invertebrates are important to defining the role of these animals in regenerating nutrients from sediments into overlying waters. In determining excretion rates in the laboratory, natural conditions should be simulated as closely as possible. Because benthic animals live in close contact with the sediments, one factor that may affect their excretion rates is whether or not a particle substrate is provided. Previous studies have shown that respiration rates of benthic invertebrates are significantly lower when a substrate is present than when one is absent (Vernberg et al. 1977; Provini and Marchetti 1976). In the few studies that have quantified excretion rates of benthic invertebrates, one has used a substrate (Gardner et al. 1981), while others have not (Johannes 1964; Hargrave and Geen 1968; Hawkins and Keizer 1982).

We examined the effect of a sand substrate (presence vs. absence) and the effect of water medium (lake water vs. distilled water) on phosphorus excretion rates of three freshwater benthic taxa: tubificids, chironomids, and the amphipod *Pontoporeia hoyi*.

Methods — Benthic organisms were collected with a Ponar grab from three different sites in southeastern Michigan. Chironomids and tubificids were collected about 3 km south of the mouth of the Grand River in August 1981 and about 1 km south of the river mouth in July 1982. Water depth at both sites was 11 m. Chironomids were identified as *Chironomus fluviatilis*-gr. and tubificids were mainly of the type without hair setae, probably of the genus *Limnodrilus*. Sediments at both sites consisted of fine sand with some detritus, with more detritus evident at the site closer to the river mouth. The amphipod *Pontoporeia hoyi* Smith was collected in early June and early July 1982 at a site 4 km west of the river mouth at a water depth of 23 m. Sediments at this site consisted of medium to coarse sand. Samples at each of these three sites were washed through a 0.6 mm screen and the organisms retained were transferred to the laboratory in their natural sediment substrates maintained at in situ temperatures. Experiments on *P. hoyi* were initiated within 6 h after collection and those on tubificids and chironomids were initiated within 24 h after collection. All experiments were conducted at in situ temperatures (5°C for *P. hoyi* and 16°C for tubificids and chironomids).

The experiments were designed to test the effects of a sand substrate and type of water medium on excretion rates. Four sets of experimental vials were prepared: substrate and lake water, substrate and distilled water, lake water alone, and distilled water alone. The substrate consisted of 2 g of washed and ignited sand (Mallenckrodt, catalog No. 7062; median grain size = 0.32 mm diameter) and the water was either low-P purified distilled water or low-P filtered (0.45 μm pore size Millipore) Lake Michigan water. The vials with and without the sand substrate were rinsed five times with the appropriate water and additional water was then added to give a total water weight of 9 g. Tubificid experiments were conducted with 7–11 animals per experimental vial, whereas chironomid and *P. hoyi* experiments had 3–4 animals per experimental vial. Control vials were identical to the corresponding experimental vials except for the absence of animals. Tubificids and chironomids were cleaned of adhering debris and rinsed before they were transferred into the experimental vials. Because *P. hoyi* is sensitive to handling, individuals of this species were transferred directly into the experimental vials without additional rinsing. As a check for material entering the experimental vials with the animals, separate *P. hoyi* individuals were momentarily placed into the control vials and then immediately removed. Incubations were terminated after 24 h by removing the animals from the vials with clean forceps. Results from experimental vials with any dead or missing organisms were not used in the rate calculations.

Inorganic phosphorus was determined by adding 1 mL of 1 mol/L NaOH to each vial (to give a 0.1 *N* solution), mixing the vial on a rotary shaker for 18 h, and then measuring soluble reactive phosphorus with a Technicon AutoAnalyzer (Gardner et al. 1981). The mean P concentration in control vials was subtracted from the concentration of P in each corresponding experimental vial to estimate the amount of P excreted by the animals during the incubation interval. A few experimental and control vials had P levels far greater than the mean of the other vials for that test condition; these vials were not used for rate determinations.

Animals from each experimental vial were dried in aluminum planchets for 24 h at 70°C and then ashed at 550°C for 1 h to determine ash-free dry weight (AFDW). Mean AFDW per individual was 1.63, 0.92, and 0.38 mg for *P. hoyi*, chironomids, and tubificids, respectively.

We tested the effects of sand (presence vs. absence) and water medium (distilled vs. lake) on excretion rates for each taxa using multivariate ANOVA techniques. Because tubificids and chironomids were collected at two different sites in 1981 and 1982, a third variable, the year of collection, was also tested in these two taxa.

Results and discussion — Mean excretion rates of P under the various experimental conditions are given in Table 1. The presence or absence of a substrate did not significantly ($P < 0.05$) affect the excretion rate of any of the three taxa.

TABLE 1. Inorganic phosphorus excretion rates (nmol P·(mg AFDW)$^{-1}$·h^{-1}) for chironomids, tubificids, and the amphipod *Pontoporeia hoyi* under the various experimental conditions. Differences were tested separately for each taxa using ANOVA. All interactions were nonsignificant.

		$\bar{x}$	(SD)	n	P
		Chironomidae (16°C)			
Sand	with	0.54	(0.33)	19	ns
	without	0.59	(0.35)	19	
Water	lake	0.60	(0.33)	19	ns
	distilled	0.53	(0.34)	19	
Year	1981	0.39	(0.19)	23	<0.001
	1982	0.83	(0.34)	15	
		Tubificidae (16°C)			
Sand	with	0.17	(0.12)	21	ns
	without	0.13	(0.13)	19	
Water	lake	0.14	(0.14)	22	ns
	distilled	0.18	(0.11)	18	
Year	1981	0.12	(0.10)	24	<0.001
	1982	0.27	(0.11)	16	
		Pontoporeia hoyi (5°C)			
Sand	with	0.08	(0.04)	18	ns
	without	0.09	(0.04)	15	
Water	lake	0.08	(0.04)	23	<0.03
	distilled	0.11	(0.03)	10	

These results contrast to previous reports of a significant "substrate-effect" on respiration rates (Vernberg et al. 1977; Provini and Marchetti 1976). The different response in the two rate functions may reflect a varying effect of short-term changes in metabolic activity. For example, when a substrate is not present many benthic organisms become more active (Vernberg et al. 1977), or become hyperactive with varying periods of rest (Pamatmat 1982). If the animals initially metabolize energy reserves that are low in phosphorus and nitrogen (fats and carbohydrates) to meet the resulting increase in metabolic demand, respiration rates could increase without a corresponding increase in excretion rates. Therefore, over the short-term, excretion rates may not reflect metabolic activity as accurately as respiration rates do, but may be more a function of the quality and quantity of food ingested. Another explanation for the different response in the two rate functions is that, since benthic animals are subjected to a certain amount of "stress" in the absence of a substrate (Chapman et al. 1982), respiration rates may simply be a better indicator of metabolic stress than excretion rates. Indeed, pollutants affected the respiration rate of tubificids (Brkovic-Popovic and Popovic 1977), but did not affect the ammonium excretion rate of cladocerans (Gardner and Miller 1981).

The type of water medium used, lake water or distilled water, did not affect the excretion rate of tubificids or chironomids, but the excretion rate of *P. hoyi* was slightly, but significantly, higher in distilled water (Table 1). It is not clear why the water medium made a difference in the excretion rate of *P. hoyi*. This species is found mainly at the sediment surface and in the water column, whereas tubificids and chironomids are found deeper in the sediments. Perhaps *P. hoyi* was more sensitive to chemical differences in the water medium than the other two taxa and thus was under more "stress" in distilled water than in lake water. To provide further insight into the possible relationship between P excretion and stress, the mortality of the three taxa under each of the experimental conditions was calculated. The mortality of tubificids and chironomids, expressed as the percentage that died in 24 h, was less than 3% for any given experimental condition. However, the mortality of *P. hoyi* in sand–lake water, lake water, sand–distilled water, and distilled water was 0, 11, 24, and 34%, respectively. Clearly, *P. hoyi* was more "stressed" in distilled water than in lake water, and also more "stressed" in the absence of a substrate than when one was present. Because the water medium had only a slight effect on P excretion in *P. hoyi* and the presence or absence of a substrate had no effect, excretion rates apparently are not an indication of "stress" in this species. Thus, the increased excretion of *P. hoyi* may have been a specific response to the distilled water rather than a general response to stress.

Although laboratory conditions (temperature, handling procedures, etc.) were the same in both years, excretion rates of both tubificids and chironomids were significantly higher in 1982 than in 1981 (Table 1). These differences were likely related to the animals being collected from a site much closer to the river mouth in 1982. Sediments near the river mouth have a higher P content than sediments from surrounding areas (Chambers and Eadie 1980) and the higher excretion rate of animals from this site may reflect this richer source of food.

Our results indicate that realistic determinations of P excretion in these three taxa can be obtained in the absence of a substrate. Although *P. hoyi* was more "stressed" in the absence of a substrate (as judged by higher mortality), rates of P excretion were not affected. Of course, excretion rates of other benthic taxa may not be similarly unaffected by the absence of a substrate, and it is recommended that substrate effects in other taxa be determined before excretion rates are measured. Our results also indicate that, at least for *P. hoyi*, "natural" water is preferable to distilled water as the aqueous medium for excretion experiments.

The elimination of a substrate greatly simplifies the experimental approach for P release determinations. To determine excretion rates accurately, P levels in control vessels (without animals) must be subtracted from P levels in experimental vessels (with animals). Ideally, control levels should be small relative to experimental levels. If a natural substrate is used, this may not always be possible. For example, we could not use lake sediments as a substrate in our experiments because P levels were too high even after several extractions (unpublished data). The use of "clean" washed sand was more acceptable, but P levels in our sand–water controls still averaged 38% of the P levels in the experimental vials containing sand, water, and animals. In contrast, P levels in control vials containing water alone were negligible. Thus, determinations of P excretion in the absence of a substrate provides greater sensitivity and makes it more feasible to measure excretion rates of individual benthic animals. The advantages of measuring excretion on individual animals have recently been discussed by Gardner and Scavia (1981) and Hawkins and Keizer (1982).

Acknowledgments — We thank W. R. Burns, J. E. Grimes, and D. V. Morse for their help in collecting animals and G. Laird for preparing the vials. We also thank L. Herche for providing the statistical analysis. P. F. Landrum and D. Scavia critically reviewed the manuscript.

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Seasonal and Diel Patterns of Emergence from the Redd of Atlantic Salmon (*Salmo salar*) Fry

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GUSTAFSON-MARJANEN, K. I., AND H. B. DOWSE. 1983. Seasonal and diel patterns of emergence from the redd of Atlantic salmon (*Salmo salar*) fry. Can. J. Fish. Aquat. Sci. 40: 813–817.

The emergence of Atlantic salmon (*Salmo salar*) fry from both artificial and wild redds was monitored on a daily basis for the duration of the emergence period by means of fry traps. On the nights of peak emergence, we made hourly checks. Emergence was largely restricted to three nights, during which most activity was confined to a 3-h period. We hypothesize that the sharp diel periodicity is due either to a stimulus–response or a clock-controlled mechanism and that synchronous nocturnal emergence is probably a predator avoidance tactic.

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À l'aide de trappes à alevins, nous avons suivi quotidiennement l'émergence d'alevins de saumon atlantique (*Salmo salar*) de sillons de ponte, tant artificiels que naturels, tout au long de la période d'émergence. Les nuits où il y avait pic d'émergence, nos observations ont été faites à toutes les heures. L'émergence s'est limitée en grande partie à trois nuits, durant lesquelles le gros de l'activité n'a duré que trois heures. Nous émettons l'hypothèse que cette périodicité nycthémérale bien définie est due, soit à une réaction à un stimulus, soit à un mécanisme dépendant de l'heure du jour, et que l'émergence nocturne synchronisée est probablement une tactique d'évitement des prédateurs.

Received August 20, 1982
Accepted February 28, 1983

Reçu le 20 août 1982
Accepté le 28 février 1983

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Printed in Canada (J7043)
Imprimé au Canada (J7043)

THE timing of emergence of Atlantic salmon (*Salmo salar*) fry from the redd is not random, but shows a definite rhythmicity. Most Atlantic salmon fry emerge under cover of darkness (Dill 1970; Jordan 1981), but no precise hourly profile of the emergence pattern has been reported. Similarly, daily records of Atlantic salmon emergence from individual redds have not been taken with sufficient accuracy to permit precise clarification of seasonal profiles. The temporal organization of emergence could be an important factor in fry survival (Ginetz and Larkin 1976; Godin 1980). This study was undertaken to determine accurately the time of fry emergence from individual redds.

Materials and methods — On February 17–18, 1981, three artificial redds were constructed in Old Stream, Wesley, Maine. We used eyed eggs, dug from wild redds at nearby sites in Old Stream, for planting a known number of live eggs in each of the three artificial redds. Dead eggs quickly turn milky, and are easily spotted and removed. Wild eggs were chosen instead of hatchery eggs to simulate wild conditions as closely as possible, eliminating the need to account for cumulative temperature effects (i.e. the total number of degree days for each batch of eggs). This also eliminated concern over the suitability of the eggs to the stream in which they were to be planted and possible transportation effects upon the eggs.

The wild redds were manually excavated with a hooded shovel in a manner similar to that described by Hobbs (1937). The eggs were swept from the excavated gravel deposited in front of a net placed downstream of the redd. The contents of the bag-net were transferred to a plastic pail filled with stream water, and the eggs were decanted off. Their number was estimated volumetrically by measuring water displacement. After approximately 1500–2000 eggs had been collected from a wild redd, they were transferred to an artificial redd.

Suitability of the artificial redd site was determined by checking permeability with a Mark VI standpipe (Terhune 1958). Permeabilities ranged from 1271 to 1510 cm/h, comparing favorably with values for natural salmon redds.

From April 29 to May 8, fry traps (Porter 1973) were set on the three artificial redds in Old Stream, as well as on one wild redd in Crooked River, and on three wild redds in the West Branch of the Machias River (all in Wesley, Maine). The water depths at the study sites then ranged from 10 to 60 cm. Mesh netting (0.6 mm) was placed over the redd and buried to a depth of approximately 30 cm around and well away from the outsides of the redd. The downstream end of the net was V-shaped with an opening leading into polyvinyl chloride tubing. This in turn connected to the trap's holding box. Emerging fry were immediately swept downstream into the trap holding box by the force of the current. To count the number of fry emerging during a given interval the trap lid was raised, and the fish were observed without disturbing the trap or handling any of the fish. After a count, the back of the trap was opened, allowing the fish to swim out.

All traps were monitored on a daily basis from April 29 until June 4, at which time the traps were removed. Repeated vandalism of one of the traps on the West Branch of the Machias prevented use of its data in the daily profile. In addition, from the evening of May 19 to the morning of May 22, the three artificial redds in Old Stream were monitored on an hourly basis from sundown until sunrise, or until fry had stopped emerging. A final check was always made at sunrise. On May 21–22, two of the wild redds in the West Branch of the Machias River were also monitored on an hourly basis. These data were pooled to yield a single 24-h profile of emergence.

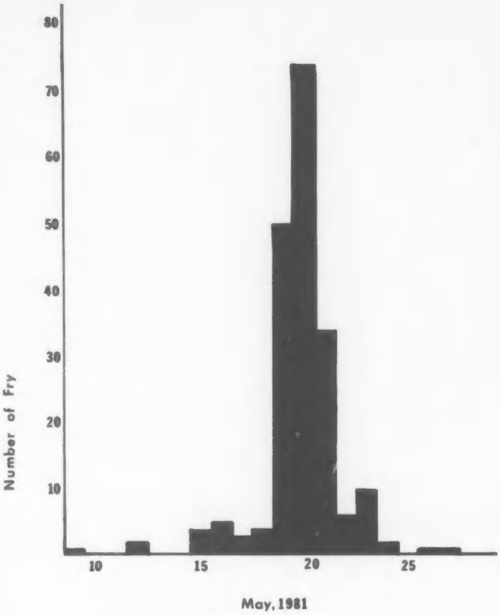

FIG. 1. Pooled data from all traps for emergence of fry on a daily basis from May 12 to May 28, 1981. Each daily peak of emergence spans 2 d, since midnight occurs roughly in the middle. To avoid splitting a given peak of emergence, data are totalled from noon to noon, and appear in the chart on the first of the 2 d. The single fish appearing on May 9 was dead, and probably had been mechanically dislodged.

Specimens for stomach content analysis were collected on the evening of May 20 from each of the three artificial redds in Old Stream. At approximately 21:00, two fish were collected from each site as soon as they appeared in the trap box, insuring that they had just emerged from the gravel. Two more fish were collected from the second of these sites at 24:00. The fish were measured and preserved in formalin.

Results — The first of the fish began to emerge on May 12, and emergence continued until May 28. Since all the individuals were comparable in size and age, data from all redds were pooled. During a 3-d period (May 19, 20, and 21), 80% of the emergence occurred (Fig. 1). Analysis of this distribution indicates a significant departure from normality. The data are skewed to the left ($g_1 = -1.21$, $P < 0.001$) and are leptokurtic ($g_2 = 7.10$, $P < 0.001$). During the three nights of hourly activity recording approximately 84% of emergence occurred between 21:00 and 24:00 (Fig. 2). This distribution

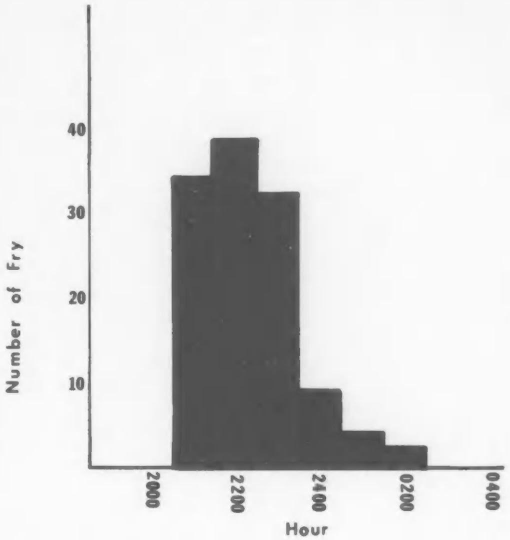

FIG. 2. Frequency histogram based on the sum totals from the pooled hourly emergence data from all traps. Data from the sunrise (05:00) check of traps are not included (see text).

is also nonnormal, being skewed to the right ($g_1 = 1.00$, $P < 0.001$) and leptokurtic ($g_2 = 0.91$, $P < 0.05$).

Thirteen fish were captured at the final sunrise check. Since their emergence could not be assigned to a specific hour, they were eliminated from both the histogram and statistical analysis of the distribution. They constitute only about 10% of the fish captured, and no matter where they are placed in the few hours of the pre-dawn morning when they must have emerged, their addition to the distribution could only serve to increase the skewness to the right and leptokurtosis.

Eight fish in all, averaging 30 mm in length, were collected from three redds. All had full stomachs. They had been feeding exclusively on black fly larvae (*Simulium* sp.), which were numerous in all the streams under observation and covered the stream beds.

Discussion — Nighttime emergence of Atlantic salmon fry was reported by Jordan (1981). This is common for salmonids. Studies by Heard (1964) and Neave (1955) have shown nighttime emergence for sockeye salmon (*Oncorhynchus nerka*) and pink salmon (*O. gorbuscha*), respectively. Godin (1980) found that about 80% of pink salmon fry emerged from simulated redds in the laboratory during the hours of darkness. Pink, sockeye, and chum salmon (*O. keta*) migrate as fry, and do so predominantly at night (Hoar 1958; Hunter 1959). McDonald (1960) also documented night migration of sockeye and coho (*O. kisutch*) salmon fry, as well as pink and chum salmon fry. The movements of pink and chum fry may extend into daylight at the end of a long migration. Rainbow trout fry (*Salmo gairdneri*) emerge and migrate downstream predominantly at night (Northcote 1962, 1969; Dill 1970; Carey and Noakes 1981). Stauffer (1972) reported nighttime migration of age 1 rainbow trout. Brown trout fry (*Salmo trutta*) undergo emergence and downstream migration during the early evening, at the onset of darkness (Cuinat and Heland 1979). Our study further confirms the absence of daytime emergence in *S. salar*, and gives an accurate hourly profile of emergence activity.

There is a strong periodicity in the data with the peak of emergence bearing a fixed phase relationship to the natural light−dark cycle. Temperature does not seem to play a role. Our records of water temperature and depth indicated that the water would slowly cool after midnight, but during peak emergence times, retained its daytime value. The maximum cooling observed was seldom more than 0.5°C. Studies by Mason (1975) and by Cuinat and Heland (1979) on the migration of coho salmon and brown trout fry, respectively, support this conclusion, because they indicate that there is no apparent correlation between water temperature and flow, and the pattern of movement they observed. Godin (1980), however, observed correlations between emergence patterns and temperature in his studies of *O. gorbuscha* in laboratory redds, but the light−dark (LD) cycle was in most cases the crucial synchronizing agent.

There are two possible mechanisms consistent with the data that might explain the sharpness and its phase relation to the natural LD cycle of the emergence peak. First, the fry may be negatively phototactic, and upon reaching a state of readiness for emergence, they may wait until darkness falls, and then immediately leave the redd. A few stragglers would presumably emerge throughout the night, but the following dawn would cut off any further activity. Emerging sockeye salmon fry are known to be negatively phototactic (Heard 1964), and negative phototaxis was reported for rainbow trout, a congener of Atlantic salmon (Northcote 1962, 1964; Dill 1970; Carey and Noakes 1981).

A second possibility is that the emergence rhythm is controlled by an endogenous oscillator, or biological clock. The existence of an autonomous oscillation can only be conclusively demonstrated by laboratory isolation of the organism from cyclic environmental influences, and by testing against well documented criteria (Pittendrigh 1954; Bünning 1973; Palmer 1976). It is nonetheless possible to infer the clock's presence from field data. The pattern of fry emergence bears a striking resemblance to that of *Drosophila* eclosion, for example, an analogous event clearly under clock control (Bünning 1935; Pittendrigh 1954). Pittendrigh's (1954) emergence profiles are both leptokurtic and skewed to the right as are ours for the fry. Use of an endogenous timekeeper allows a degree of advance preparation not possible when an organism reacts directly to changing environmental conditions (Enright 1970; Hoffman 1976; Daan 1981). Employing laboratory redds such as Godin's (1980) would allow the question to be settled.

On the three nights studied, 78% of the fry emerged between the hours of 20:00 and 23:00. The reasons for maintaining such a sharp peak in emergence are not immediately obvious. Emergence under cover of darkness would reduce the sight predation upon newly emerged and weakly swimming fry, yet if predator avoidance was solely responsible for night emergence, a normal distribution would be expected.

An additional point to consider is that overall predator damage is reduced when prey concentration is high (Brown 1975; Peterman and Gatto 1978). If all the fry emerged in synchrony, their concentration would be maximized, and the percentage of the total population lost to predation would be somewhat lower owing to this concentration effect (Daan 1981). Hunter (1959) found that predation loss in groups of 100 marked and released chum salmon fry was lowest when the overall concentration of migrating fry was highest.

Neave (1955) studied the emergence patterns of pink salmon fry, which begin migrating downstream to the sea immediately upon leaving the redd. Ninety percent of the fish appeared at a weir approximately 1.6 km downstream of the redd by 02:00. Neave (1955) suggests that for pink salmon, this strategy would enable the fish to evacuate the stream in the shortest period of time, under the cover of darkness, which would greatly reduce predator pressure. *Salmo salar*, as has been noted, do not migrate downstream upon emergence, but are territorial within the area of the redd (Kalleberg 1958; Keenleyside and Yamamoto 1962, 1976; Symons 1974, 1976; Wankowski and Thorpe 1979), although downstream displacement can occur, depending on the water level and the position of the redd within the stream (Ottaway and Clarke 1981). Symons (1974) has documented a reduction in predation of young Atlantic salmon by brook trout (*Salvelinus fontinalis*) when the fish had established territories. Emerging early in the evening could give the fry more time to settle to the bottom in order to hold station, and avoid further displacement downstream. By sunrise, the fry could be well situated in territories within the stream.

The fry are apparently not emerging in synchrony with increased invertebrate drift. All of the stomachs we checked contained black fly larvae, which solidly covered the stream bed. Waters' (1962) study showed no diurnal periodicity of black fly larvae in a stream, despite nightly increases of drift for many other invertebrate species. Williams (1981) showed that Atlantic salmon fry would feed on whatever was most abundant, with no preference. In our study, fry emerging from the redds apparently found the numerous black fly larvae covering the stream substrate to be the most easily available food.

The sharpness of the emergence peak in the seasonal profile was unexpected. The preponderance of emergence activity was confined to only three nights, indicating some nonrandom mechanism is at work. The leptokurtosis seen is indicative of an excess of values near the mean and in the tails, compared to a normal curve (Snedecor and Cochran 1967).

No mechanism is immediately obvious that could be a cue. The water temperature was rising gradually throughout the period, but there is no sharp threshold that might be a trigger. Synchronous exhaustion of yolk supplies is also unlikely as the trigger. While sibling fry might use up all their yolk at about the same time, thus forcing emergence, each of our artificial redds contained eggs from several natural redds, were thus from different females, and were spawned on different days. Also, we pooled data from artificial and natural redds found in different streams. Additionally, the fry had access to *Simulium* larvae within the gravel substrate, and those examined had clearly fed well on them before emergence. Some external cue accessible to all the fry must be involved to allow the synchrony we recorded.

One possibility for further investigation is lunar synchrony. Full moon occurred on May 18, just before the three day peak of activity, but no conclusions can be drawn from the coincidence of two events during a single cycle. Grau et al. (1981) have demonstrated a strong correlation between lunar phase and a surge in thyroxine during pre-migratory smoltification in hatchery-reared coho salmon. In this instance, however, the new moon seemed to be the cue, allowing seaward migration during the darkest nights of the month, presumably a predator avoidance tactic. The night of maximum emergence of sockeye salmon for Heard's (1964) data was on June 16, 2 d after new moon. If a lunar cue is functioning in the emergence reported here, the reasons for having the fry leave the redd on the brightest nights of the month are unclear. Ginetz and Larkin (1976), for example, found that predation on migrant sockeye salmon fry increased as light levels increased from cloudy night levels to moonlit night levels.

Whatever the mechanism, there does appear to be synchrony in the population on a seasonal level. Again, this synchrony would serve to keep the concentration of fry higher than a random process could, and might be serving to reduce overall predation. Further investigation may elucidate other ecological benefits.

Acknowledgments — This research was partly funded by the International Atlantic Salmon Foundation, the Maine Department of Inland Fisheries and Wildlife, and the U.S. Fish and Wildlife Service. We are grateful to Dianne Barker for invaluable field assistance, A. L. Meister of the Atlantic Sea Run Salmon Commission of Maine for advice and equipment, and R. M. Jordan of the Maine Department of Inland Fisheries and Wildlife and K. Beland of the Salmon Commission for helpful advice in construction of the artificial redds. We are also indebted to Drs J. McCleave, J. Moring, and J. Ringo of this Department for assistance in the preparation of this manuscript, and to P. Dowse for the illustrations.

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Assessment of Phosphorus Limitation in an Oligotrophic Lake Using Radiophosphorus Uptake Kinetics[1]

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Chow-Fraser, P., and H. C. Duthie. 1983. Assessment of phosphorus limitation in an oligotrophic lake using radiophosphorus uptake kinetics. Can. J. Fish. Aquat. Sci. 40: 817–821.

We used ^{32}P uptake kinetics as a means of assessing periods of P limitation to phytoplankton growth in an ultraoligotrophic lake. Tracer experiments, conducted biweekly and weekly in 1975 and 1976, respectively, yielded information regarding the availability of phosphorus over the sampling seasons. Analytical measurements of ambient phosphorus concentrations were not as informative as uptake kinetic data in the assessment of phosphorus demands in the lake. Whereas analytical methods identified probable periods of P limitation at the end of summer, radiophosphorus data indicated that P limitation was prevalent throughout most of the summer.

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Printed in Canada (J6814)
Imprimé au Canada (J6814)

CHOW-FRASER, P., AND H. C. DUTHIE. 1983. Assessment of phosphorus limitation in an oligotrophic lake using radiophosphorus uptake kinetics. Can. J. Fish. Aquat. Sci. 40: 817–821.

La cinétique de l'absorption de ^{32}P a servi à évaluer les périodes durant lesquelles le phosphore est le facteur limitatif de croissance du phytoplancton dans un lac ultra-oligotrophe. Des essais avec marqueurs, effectués à intervalles bihebdomadaires en 1975 et hebdomadaires en 1976, ont fourni des données sur l'accessibilité du phosphore pendant les saisons d'échantillonnage. Les mesures de concentrations du phosphore ambiant n'ont pas donné autant d'information que la cinétique de l'absorption dans l'évaluation de la demande en phosphore dans le lac. Alors que les méthodes analytiques permirent d'identifier des périodes probables de limitation du phosphore à la fin de l'été, celle du phosphore radioactif indiqua que la limitation du phosphore prédominait durant la majeure partie de l'été.

Received February 12, 1982
Accepted March 8, 1983

Reçu le 12 février 1982
Accepté le 8 mars 1983

UNTIL recently, assessment of phosphorus demands in lakes has been limited to the correlation of conventional characteristics such as total phosphorus (TP), soluble reactive phosphorus (SRP), or soluble unreactive phosphorus (SUP) to the phytoplankton biomass or chlorophyll *a*. This approach produced results that were difficult to interpret and often misleading. One explanation for this inaccuracy has been attributed to the inappropriateness of employing a static measurement to describe a dynamic system that exhibits a constant flux among various P compartments (Lean 1973a, 1973b; Lean and Rigler 1974; Halfon 1979). Static measurements, in addition to being inappropriate, have also been problematic when applied to ultraoligotrophic systems. The forms of P exist in such low concentrations that they often escape detection or are inaccurately measured owing to sensitivity limitations. Also, some organic P may be hydrolyzed by acidic reagents, giving overestimates of SRP (Rigler 1968; Downes and Paerl 1978; White et al. 1981). A dynamic system is more appropriately measured by a kinetic term (e.g. the "turnover time" of an uptake curve) that indicates the rate of P uptake.

Lean and Nalewajko (1979) were able to use ^{32}P uptake experiments to assess periods of P limitation in small and large lakes, and Levine and Schindler (1980) measured seasonal changes in the flux of orthophosphate to seston in two Canadian Shield lakes. Recently, White et al. (1982) identified factors affecting orthophosphate turnover times in New Zealand and Canadian lakes. In none of these studies are turnover times compared with parallel changes in phytoplankton abundance.

The objective of our study was to test the effectiveness of P kinetics for assessing P demand in an ultraoligotrophic lake. We were particularly interested in comparing P kinetics and standard analytical methods to observed changes in phytoplankton abundance in hopes that kinetics may be used as an alternate means for the identification of P limitation in oligotrophic systems.

Lake Matamek (65°50′W, 50°20′N) is a Precambrian Shield lake with an area of 1320 ha and a maximum depth of 100 m (Janus and Duthie 1979a). The lake is acidic, with a summer pH of 5.5–6.0, and is very low in dissolved solids. Conductivity is in the range 10–20 μmho/cm, and total cations do not exceed 4 mg/L. The mean summer chlorophyll *a* concentration of about 1.5 mg/m^3 and the summer phytoplankton biomass of generally between 100 and 150 mg/m^3 attest to its ultraoligotrophic nature. The phytoplankton and primary productivity of Lake Matamek have been described (Janus and Duthie 1979b; Ross and Duthie 1981), and a P budget has been calculated (Chow-Fraser and Duthie 1983).

Methods — Biweekly $^{32}PO_4$ uptake experiments were carried out during 1975 at a central station in Lake Matamek (Matamek Centre) from the beginning of June until late August. Surface water samples were brought back to the laboratory within 30 min of collection. Samples were also taken for SRP and phytoplankton biomass determinations. The SRP and TP samples were obtained from a depth of 0.5 m, and the integrated phytoplankton samples were collected by means of a 2-m-long rubber hose lowered vertically into the water. In 1976 the same sampling methodology was carried out weekly at a station approximately 2 km to the east (East Basin) of Matamek Centre.

The method of Lean (1973b) was used to estimate P turnover times. To a 600-mL sample of lakewater was added 9.25 MBq of carrier-free $^{32}PO_4$. At various time intervals, 10-mL aliquots were withdrawn and immediately filtered through 0.45-μm pore size membrane filters. The filters were rinsed with 20 mL of distilled water and then placed in scintillation vials. All incubations were carried out for 4-h periods. The logarithm of the percentage of total radioactivity remaining in the filtrate was plotted against time. The reciprocal of the initial slope of the curve yielded the turnover time. Samples for TP (in 1976) and SRP (in 1975) were analyzed by the methods in Strickland and Parsons (1972). Phytoplankton enumeration was carried out using an inverted microscope and freshweight biomass estimated by the method described by Janus and Duthie (1979a).

Results and discussion — Interpretation of radiophosphorus uptake curves — Figure 1 shows representative uptake curves that were obtained in Lake Matamek over the two sampling years and describes approximately 75% of our observations. The three curves correspond to types described by Lean and Nalewajko (1979). Figure 1a corresponds to the

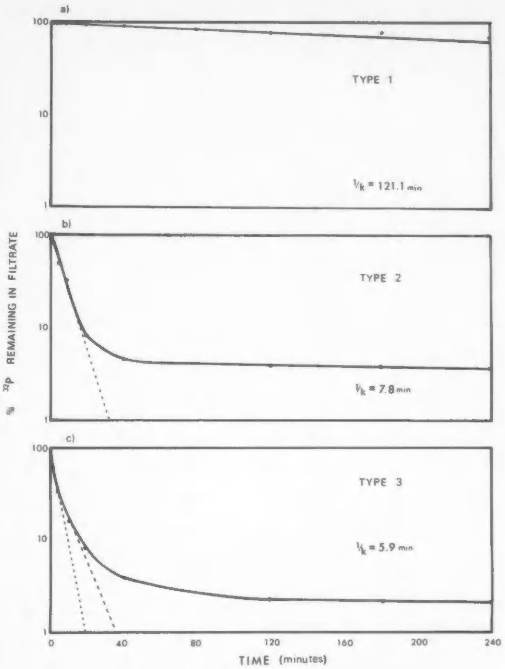

FIG. 1. Examples of uptake curve types 1, 2, and 3 (Lean and Nalewajko 1979). Type 1 (9 June 1975, Matamek Centre); type 2 (20 July 1976, East Basin); type 3 (4 August 1975, Matamek Centre).

type 1 curve; the long turnover time of 121.1 min suggests that P was abundant or in low demand. Figure 1b corresponds to the monophasic type 2 curve where P is depleted and/or in high demand. The kinetics can be described using a two-compartment model: phosphate exchanging with cellular P with insignificant formation of organic P. Figure 1c, however, corresponds to Lean and Nalewajko's (1979) diphasic type 3 curve thought to represent extreme P depletion or very high demand and occurs at times when colloidal P and a phosphorus compound (XP) of low molecular weight become labeled in less than 1 min.

Figure 2a appears to be a transition type between type 1 and type 2 (type 1−2). The initial portion of the curve slopes very gently and is followed by a gradual asympote. In this particular case there is a possibility that even after 4 h of incubation, the system has not reached an asymptotic level. Figure 2b is an example of a curve that cannot be explained using a two-compartment model even though the curve is log-linear. Were it not for the relatively low turnover time this curve could be mistaken for type 1 kinetics. However, a turnover time of 6.9 min implies a very rapid incorporation of P, characteristically associated with a P-depleted condition. Figure 2c presents yet another situation in which the uptake kinetics appear to be intermediate between two curve types. The oscillation of points about the asymptote renders the task of distinguishing between curve types 2 and 3 extremely difficult.

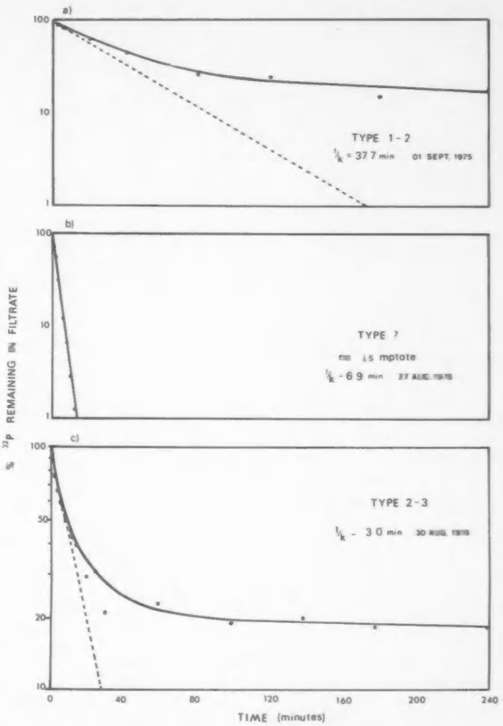

FIG. 2. Examples of transitional P uptake curves. Type 1−2 (1 September 1975, Matamek Centre); type ? (27 August 1976, East Basin); type 2−3 (30 August 1976, East Basin).

Paramount to a proper understanding of P demands in lakes is the recognition that there is a constant exchange of P between two or more compartments. Our suggestion that there are transition types between the types of uptake curves described by Lean and Nalewajko (1979) is totally in keeping with the dynamic nature of the model.

Interpretation of P limitation— Figure 3 summarizes the seasonal variations in uptake curve types for Matamek Centre in 1975 and corresponding measurements of turnover times, SRP concentrations, and algal biomass. In the beginning of June, both biomass and SRP measurements were relatively high. Both decreased until early July and then increased again, but by mid-August the curves had diverged, with SRP being minimal and biomass being maximal. Since algal biomass was high and SRP was very low in August, P limitation was likely. The low biomass and relatively high SRP values from June to mid-July could be interpreted to mean a non-P-limiting system.

The type 2 curve and the relatively rapid turnover time in the early part of June imply the existence of a moderately depleted system; the type 3 curves and low turnover times

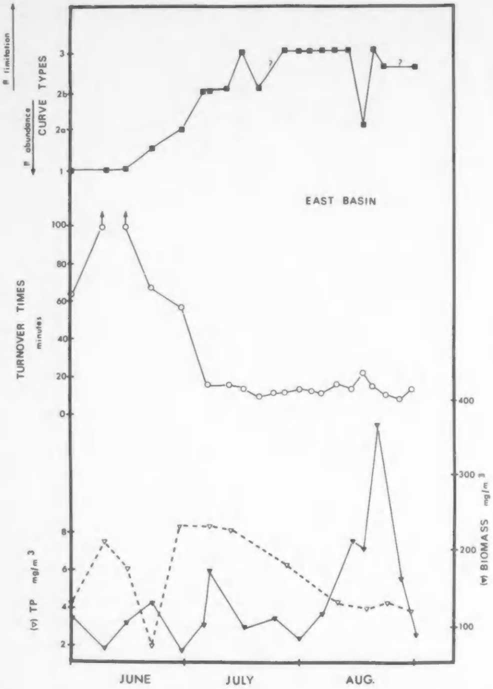

FIG. 3. Summary of uptake curve types, turnover times, soluble reactive phosphorus concentrations (SRP), and phytoplankton biomass concentrations (biomass) for Matamek Centre, 1975.

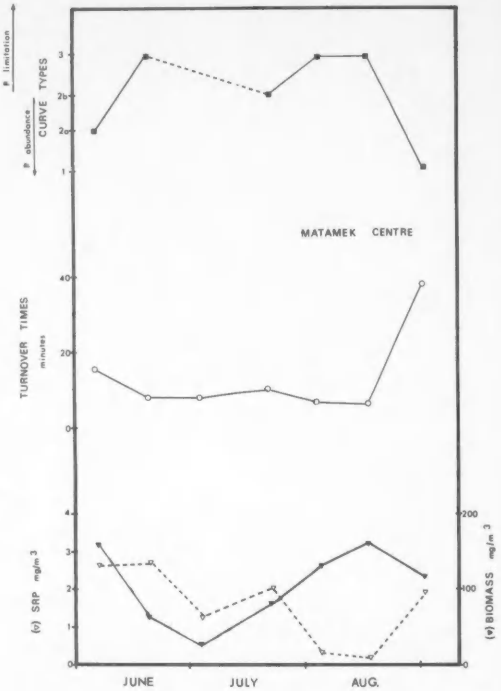

FIG. 4. Summary of uptake curve types, turnover times, total phosphorus concentrations (TP), and phytoplankton biomass concentrations (biomass) for East Basin, 1976.

exhibited from mid-July to mid-August also corroborate with the interpretation of SRP that the system was P limited. However, interpretations of the kinetic data diverge from those of SRP measurements from mid-June to mid-July. Whereas the types 3 and 2 together with corresponding low turnover times were indicative of P limitation, SRP and biomass measurements were not. In summary, while uptake kinetics revealed that Matamek Centre was P limited, or at least P depleted for most of the summer in 1975, analytical measurements implied limitation only at the end of the summer.

The data for the East Basin is summarized in Fig. 4 and may be interpreted in a fashion similar to those for Matamek Centre. The inverse relationship between TP and algal biomass in June and August is clear. These trends suggest that initially there was an abundance of P, probably as a result of spring overturn, but as biomass increased, P became depleted and the system was probably P limited by late June. The very high algal biomass and low TP measurements in mid-August also imply P limitation.

With reference to the corresponding P uptake kinetic data for June, the type 1 curve and extremely high turnover times (in excess of 100 min) substantiate our interpretation that P was not in high demand. A more concentrated sampling effort in East Basin permitted the observation of the transition curve type 1−2. By late June, the occurrence of type 2 and shorter turnover times indicated that P was in higher demand. In mid-August, transition type 2−3 and type 3 curves, together with extremely shortened turnover times and high algal biomass, strongly indicated a very high P demand in the lake. Up to this point, uptake kinetics in East Basin concur with the interpretation reached by conventional measurements. The discrepancy occurs in the period between late June and early August. Type 2 and 3, in conjunction with very short turnover times, are indicative of a very high P demand, not revealed by TP and algal biomass measurements. As in Matamek Centre, the kinetic data inferred that P was in high demand throughout most of the summer in 1976, even though conventional measurements did not.

The foregoing comparison of algal biomass, analytical, and kinetic data points out the interpretive difficulties with measurements of SRP and TP. These appear adequate for indentifying P limitation during periods of low P concentrations, but can be misleading during other periods. The basic problem is that a P measurement, regardless of the algal biomass, can be equally associated with a high or low P demand. Therefore, the moderately high SRP and TP measurements in midsummer in Matamek Centre and East Basin could not accurately reflect the P-limited condition in the lake. On the other hand, corresponding kinetic data were capable of describing the high P demand in both instances. Seasonal trends of the measured parameters are more important than their respective mean values when assessing P availability in a particular lake. The use of uptake curve types to distinguish the degree of P demand at specific times enhances the sensitivity of using turnover times alone.

Acknowledgments — The research was supported by grants to H. C. Duthie from the Woods Hole Oceanographic Institution and from the Natural Sciences and Engineering Research Council of Canada. G. McLachlan and I. Jordan provided field assistance. We thank W. D. Taylor for a critical reading of the manuscript.

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Eubothrium salvelini (Cestoda: Pseudophyllidea) Impairs Seawater Adaptation of Migrant Sockeye Salmon Yearlings (*Oncorhynchus nerka*) from Babine Lake, British Columbia

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Migrant sockeye salmon yearlings (*Oncorhynchus nerka*) were captured at the outlet of Babine Lake, British Columbia, in 1979 and 1980 and transported to the laboratory for evaluation of their seawater adaptability in a 24-h challenge test. Fish infected with the cestode *Eubothrium salvelini* had a reduced ability to adapt to salt water, as evidenced by greater mortality and elevated plasma sodium levels after challenge. The prevalence of infection was 60% in 1979 and 30% in 1980. In 1979, mortality during challenge was significantly higher among infected than among noninfected fish; the elevation of plasma sodium levels in the infected fish was not statistically significant. In 1980, both infected and noninfected fish had improved seawater adaptability compared with the previous year; infected fish did not suffer significantly higher mortality after challenge but their plasma sodium levels were elevated significantly compared with the noninfected fish. The reduced seawater adaptability of infected fish is likely to reduce their ocean survival considerably.

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Printed in Canada (J7018)
Imprimé au Canada (J7018)

BOYCE, N. P., AND W. C. CLARKE. 1983. *Eubothrium salvelini* (Cestoda: Pseudophyllidea) impairs seawater adaptation of migrant sockeye salmon yearlings (*Oncorhynchus nerka*) from Babine Lake, British Columbia. Can. J. Fish. Aquat. Sci. 40: 821–824.

Des saumons nerka (*Oncorhynchus nerka*) migrateurs de l'année ont été capturés à l'émissaire du lac Babine (C.-B.) en 1979 et 1980, et transportés au laboratoire en vue d'évaluer leur adaptabilité à l'eau de mer dans des essais de 24 h. Les poissons parasités par le cestode *Eubothrium salvelini* eurent plus de difficulté à s'adapter à l'eau salée, comme le démontrent une plus forte mortalité et des niveaux de sodium plasmatique élevés après les essais. L'incidence d'infestation était de 60 % en 1979 et 30 % en 1980. En 1979, la mortalité lors de l'exposition à l'eau salée a été nettement plus élevée chez les poissons parasités que chez les poissons non parasités; l'élévation des niveaux de sodium plasmatique chez les premiers n'était pas significative. En 1980, l'adaptabilité des poissons, parasités ou non, s'était améliorée par rapport à l'année précédente; les poissons parasités ne subirent pas de mortalité significativement plus élevée après exposition, mais leurs niveaux de sodium plasmatique étaient nettement plus élevés que ceux des poissons non parasités. L'adaptabilité à l'eau de mer diminuée chez les poissons parasités réduit probablement pour la peine leur survie en mer.

Received July 2, 1982
Accepted March 11, 1983

Reçu le 2 juillet 1982
Accepté le 11 mars 1983

BABINE Lake, British Columbia, is an important nursery for sockeye salmon (*Oncorhynchus nerka*) in their 1st yr, with an annual output of 30–70 million yearlings. Approximately 30% of these yearlings are infected with the intestinal cestode *Eubothrium salvelini* with worm loads (worm weight/(fish weight − worm weight) × 100) up to 10% (Smith 1973).

The parasite is acquired by fry shortly after they enter the lake from their natal streams. The parasites develop to ovigerous adults by the time of seaward migration 1 yr later. Infected yearlings are typically smaller, less vigorous, and exhibit poorer performance in stamina tests than their noninfected counterparts (Smith 1973; Boyce 1979). Davis and Shand (1978) reported that one-third of Babine yearling sockeye smolts tested exhibited high plasma sodium levels after 24 h in salt water and failed to survive long-term exposure to salt water. This proportion corresponded to the prevalence of *Eubothrium* parasitism. In this study we examine the effects of *Eubothrium* infection on adaptation to salt water.

TABLE 1. Mortality among infected and noninfected sockeye yearlings (*O. nerka*) during a 24-h seawater challenge (effect of infection on mortality after challenge tested by chi-square). NS = not significant.

	N	*N* (%)	*P*
1979			
Exp. 1			
Infected	68	39(57.3)	
Noninfected	46	4(8.7)	<0.001
Freshwater control	165	0(0)	
Exp. 2			
Infected	74	47(63.5)	
Noninfected	51	8(15.7)	<0.01
Freshwater control	80	6(7.5)	
1980			
Infected	28	4(14.3)	
Noninfected	66	4(6.1)	NS
Freshwater control	75	0(0)	

Materials and methods — In May 1979, migrant sockeye salmon yearlings were captured by fixed fan trap at the outlet of Babine Lake, B.C., and assembled in floating pens nearby; of these, 350 were collected and transported by vehicle and aircraft to holding tanks at the Pacific Biological Station, Nanaimo, B.C. On May 30, a sample of 114 fish was tested for hypoosmoregulatory capacity and 165 were retained in freshwater as controls. On June 5, a second sample of 125 fish was challenged and 80 were retained as freshwater controls. In 1980, 200 yearlings were collected and transported as in 1979, 94 were challenged, and 75 were maintained in freshwater as controls.

Hypoosmoregulatory capacity was assessed using a 24-h seawater challenge test (Clarke and Blackburn 1977; Clarke 1982). After 2–8 d of acclimation to the laboratory, fish were transferred directly into a salinity of 30‰ at their acclimation temperature; after 24 h, blood samples were taken and plasma sodium levels were measured by flame emission spectrometry. After challenge, fish that have acquired the hypoosmoregulatory capacity for living in salt water typically show plasma sodium levels lower than 170 mmol/L, whereas fish that have not completed their physiological preadaptation to salt water suffer a substantial increase in plasma sodium levels (Clarke and Blackburn 1977). Following blood sampling, fish were measured (fork length), weighed, and examined for *E. salvelini*. Other parasites are found in this stock but their impact on the health of the fish is insignificant compared with that of *E. salvelini* (Boyce and Behrens Yamada 1977).

Results — The infected fish had a reduced ability to adapt to salt water, as evidenced by greater mortality and elevated plasma sodium levels in the challenge tests. The prevalence of infection was 60 and 59% in the 1979 tests and 30% in the 1980 test. In 1979, the rate of mortality during seawater challenge was significantly higher among infected than among noninfected fish (Table 1). Infected fish that survived the challenge had plasma sodium levels 8 and 12 mmol/L

TABLE 2. Correlation between plasma sodium concentration after a 24-h seawater challenge and fork length for Babine Lake sockeye salmon yearlings. *Differs from infected ($P < 0.025$, one-way ANOVA); **differs from infected ($P < 0.005$, one-way ANOVA).

Group	N	Mean (±SE) fork length (cm)	Mean plasma sodium (mmol/L)	Correlation of length/plasma sodium	
				r	P
Exp. 1					
Infected	29	7.3±0.07	178.3±4.1	−0.44	<0.05
Noninfected	41	7.7±0.05**	170.2±2.9	−0.42	<0.01
Exp. 2					
Infected	22	7.4±0.09	191.4±4.6	−0.39	<0.1
Noninfected	38	7.7±0.06*	179.5±4.5	−0.32	<0.05
Exp. 3					
Infected	24	7.4±0.09	173.1±3.6	0.01	>0.1
Noninfected	62	7.8±0.07**	163.0±1.1	0.07	>0.1

higher than did noninfected fish in the 1979 tests, but these differences were not significant (Table 2).

The 6-d holding period between the two tests in 1979 was followed by a decrease in seawater adaptability of both infected and noninfected fish, as indicated both by increased mortality and a greater elevation of plasma sodium after challenge. Five of the six mortalities in the freshwater control group were infected fish.

In 1980, both infected and noninfected fish had improved seawater adaptability compared with the previous year; mortality during challenge was low in both groups (Table 1) but plasma sodium levels were elevated significantly in infected fish compared with noninfected fish ($P < 0.05$, one-way ANOVA).

The noninfected fish were approximately 5% longer than the infected fish. Hypoosmoregulatory performance was related not only to parasitism but also to fish size in 1979; a significant inverse correlation between fork length and plasma sodium occurred in three of the four groups (Table 2). In 1980, there was no significant correlation between fork length and plasma sodium concentration.

Discussion — The results of all three experiments indicate that *E. salvelini* can compromise considerably the ability of yearling sockeye to adapt to salt water. Infected fish had high mortality rates in 1979 and elevated plasma sodium levels in 1980.

Because, as expected, infected fish were smaller than the noninfected fish, it is important to establish whether seawater adaptability is simply a function of size alone (see review by Wedemeyer et al. 1980). While in 1979 it was not possible to separate fish size from infection status as a factor in seawater adaptation, in 1980 there was a significant difference in hypoosmoregulatory capacity between infected and noninfected fish, which was clearly unrelated to fish size. It is reasonable to conclude, therefore, that *E. salvelini* infection can have a deleterious effect on the capacity of sockeye yearlings to adapt to salt water, quite apart from its effect on fish size. This conclusion regarding the effect of fish size on seawater adaptation is in keeping with the report by Davis and Shand (1978) who found no clear relationship between fish weight and plasma sodium in Babine yearling sockeye.

The generally poorer hypoosmoregulatory performance demonstrated in 1979 indicates that these fish were somewhat lacking in vigor or were at a less advanced stage of physiological development at the time of sampling, compared with the 1980 collection.

There are few reports on the effects of infectious disease on the adaptation of anadromous fish to salt water. Frantsi et al. (1975) reported that Atlantic salmon (*Salmo salar*) with advanced BKD experienced higher mortality during experimental exposure to salt water than did noninfected or lightly infected subjects. The gill parasite *Ichthyobodo necator* (syn. *Costia necatrix*) was reported to reduce the marine survival of juvenile chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) released from hatcheries, presumably by causing physical damage to the gills (Wedemeyer et al. 1980).

Since the gills and kidney are known to be closely associated with osmoregulatory function, it is conceivable that infection-induced impairment of these organs, as we have suggested, could adversely affect the development of hypoosmoregulatory capacity. On the other hand, the deleterious effect of *E. salvelini* infection results from competition with its host for nutrients in the intestine, producing a condition akin to starvation (Boyce 1979). This demand for nutrients might be particularly heavy during the interval between spring thaw and seaward smolt migration, which is a period of major growth and egg production in *E. salvelini* (Boyce 1974). During this period, the physiological preparations for seaward migration also render the fish more susceptible to stress (see Wedemeyer et al. 1980). The coincidence of these two events must tax severely the resources of the host fish and as a consequence, reduce its hypoosmoregulatory capacity.

The rate of mortality of the Babine Lake outmigrants that are not fully prepared to enter salt water can only be speculated upon, given the current lack of knowledge regarding the timing and behavior of these fish as they approach the sea. Possibly, some migrants could complete seawater adaptation in the course of their seaward journey (400 km). Death of nonadapted fish entering salt water could occur directly through osmoregulatory failure or indirectly through predation. Nonadapted fish would require a more extensive osmoregulatory adjustive period following entry to salt water. Accordingly, they may be less vigorous and therefore more susceptible to predation (Houston 1961). It is also possible that nonadapted fish may prolong their stay in the estuary, where they might be more readily available to predators. This reduced seawater adaptability together with their smaller size (Smith 1973) will likely decrease significantly the marine survival of infected smolts.

Acknowledgments — We are indebted to J. Blackburn, N. B. F. Cousens, J. Steube, and D. Whitaker for technical assistance and to Dr L. Margolis for helpful suggestions on the manuscript.

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Water Quality Modifies Uptake of Waterborne Methylmercury by Rainbow Trout, *Salmo gairdneri*

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RODGERS, D. W., AND F. W. H. BEAMISH. 1983. Water quality modifies uptake of waterborne methylmercury by rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 40: 824–828.

We measured the efficiency of uptake of waterborne methylmercury relative to oxygen consumption for rainbow trout, *Salmo gairdneri*, in hard or soft water and during exposure to sublethal concentrations of mercuric chloride or zinc sulphate. The relative efficiency of methylmercury uptake in soft water was more than double that measured in hard water. When mercuric chloride was added with waterborne methylmercury, uptake efficiency was further increased, with similar values obtained in hard and soft water. In contrast, addition of zinc sulphate decreased the relative efficiency of methylmercury uptake. Water quality thus significantly affects the accumulation of waterborne methylmercury by fish. In particular, calcium-dependent changes in gill permeability may explain elevated methylmercury residues observed in fish from lakes of low alkalinity and pH.

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Printed in Canada (J7183)
Imprimé au Canada (J7183)

RODGERS, D. W., AND F. W. H. BEAMISH. 1983. Water quality modifies uptake of waterborne methylmercury by rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 40: 824–828.

Nous avons mesuré l'efficacité d'absorption du mercure-méthyl en solution dans l'eau par rapport à la consommation d'oxygène par la truite arc-en-ciel, *Salmo gairdneri*, dans de l'eau dure ou douce et au cours d'expositions à des concentrations sublétales de chlorure mercurique ou de sulfate de zinc. L'efficacité relative d'absorption de mercure-méthyl dans l'eau douce est plus du double de celle observée en eau dure. Si l'on ajoute du chlorure mercurique au mercure-méthyl contenu dans l'eau, il y a augmentation encore plus forte d'efficacité d'absorption, les valeurs obtenues en eau dure et en eau douce étant identiques. Par contre, l'addition de sulfate de zinc a pour effet de diminuer l'efficacité relative d'absorption de mercure-méthyl. La qualité de l'eau influence donc de façon significative l'accumulation par les poissons de mercure-méthyl de l'eau. En particulier, les changements de perméabilité des branchies, dépendant du calcium, peuvent expliquer l'augmentation des résidus observée chez des poissons provenant de lacs de bas pH et alcalinité.

Received December 29, 1982
Accepted March 10, 1983

Reçu le 29 décembre 1982
Accepté le 10 mars 1983

FISH containing methylmercury at concentrations in excess of 0.5 mg·kg wet wt^{-1} have been reported from a number of watersheds in Canada and other industrialized nations (Armstrong 1979; Huckabee et al. 1979). While many of the waters with contaminated fish have obvious natural or industrial sources of mercury, others are without apparent sources of added mercury (Armstrong 1979). A variety of water quality factors including calcium concentration, alkalinity, and pH have been implicated as modifying factors affecting methylmercury accumulation by fish, with fish from watersheds of low alkalinity and pH containing significantly higher mercury concentrations than fish from adjacent watersheds of higher alkalinity and pH (Jernelov et al. 1975; Scheider et al. 1979; McFarlane and Franzin 1980). These factors could affect methylmercury concentrations in fish either through direct effects on the uptake and depuration of methylmercury by the fish or through indirect effects on the levels and distribution of methylmercury in the aquatic environment (Miller and Akagi 1979; Tomlinson et al. 1980).

Fish accumulate methylmercury as a result of efficient uptake from food and water, coupled with a very slow rate of depuration (Huckabee et al. 1979). Uptake of waterborne methylmercury by freshwater teleosts occurs almost entirely across the gills (Olson et al. 1973) and is directly proportional to metabolic rate and methylmercury concentration (Boddington et al. 1979; Rodgers and Beamish 1981). Gill permeability to water and ions is markedly affected by ambient water quality, particularly calcium concentration (Ogawa 1974; Isaia and Mansoni 1976; McWilliams and Potts 1978) and the presence of waterborne xenobiotics such as anionic detergents (Jackson and Fromm 1977). Natural waters of low alkalinity, hardness, and pH typically have low calcium concentrations and often have elevated metal concentrations (National Research Council of Canada 1981), factors that might also affect uptake across the gills of waterborne xenobiotics such as methylmercury. Our experiments investigated the effects of water hardness, mercuric chloride, and zinc sulphate on uptake of waterborne methylmercury by rainbow trout (*Salmo gairdneri*).

Materials and methods — Juvenile rainbow trout were maintained at 10°C in either hard (total hardness 385 ± 5 mg·L^{-1} as $CaCO_3$, pH 7.9 ± 0.1) or soft water (30 ± 3 mg·L^{-1} as $CaCO_3$, pH 7.6 ± 0.1) for at least 2 wk prior to an experiment. A detailed description of the chemical characteristics of the hard ground water is available elsewhere (Dixon and Sprague 1981). Soft water was made by proportional mixing of ground and deionized water.

The experimental procedures used to obtain simultaneous measurements of oxygen consumption and uptake of waterborne methylmercury have been previously described in detail (Rodgers and Beamish 1981). Briefly, the trout were forced to swim (30 cm·s^{-1}) in respirometers supplied with aerated water at the temperature and hardness to which the fish were acclimated. Oxygen consumption of each fish was determined for three successive 1-h intervals. Immediately preceding the closure of the respirometer for the third determination of oxygen consumption, labeled methylmercury plus 0.5 mL of a 200 mg·L^{-1} solution of $HgCl_2$ (in 0.5 mol H_2SO_4·L^{-1}) was added to three of the six respirometers. The remaining three respirometers were injected with labeled methylmercury plus 0.5 mL of 0.5 mol H_2SO_4·L^{-1}. In addition, four fish were tested in hard water to which 1.0 mL of a 1000 mg·L^{-1} solution of $ZnSO_4$ was added with the labeled methylmercury.

Water samples were taken at the beginning and end of the 1-h interval, and methylmercury concentration was determined by gamma spectrometry of ^{203}Hg (Rodgers and Beamish 1981). Inorganic mercury and zinc were determined by cold vapor atomic absorption spectrophotometry and differential pulse polarography, respectively. Trout were removed from the respirometer after 1 h of exposure to labeled methylmercury, and methylmercury uptake was determined by whole-body gamma spectrometry (Rodgers and Beamish 1981) within 24 h of the experiment. Uptake of waterborne methylmercury by nonrespiratory surfaces accounts for less than 10% of total uptake (Boddington et al. 1979), and incorporated methylmercury is depurated from fish of experimental size as a single compartment with a biological half-life in excess of 50 d (deFreitas et al. 1975). Thus, correction was

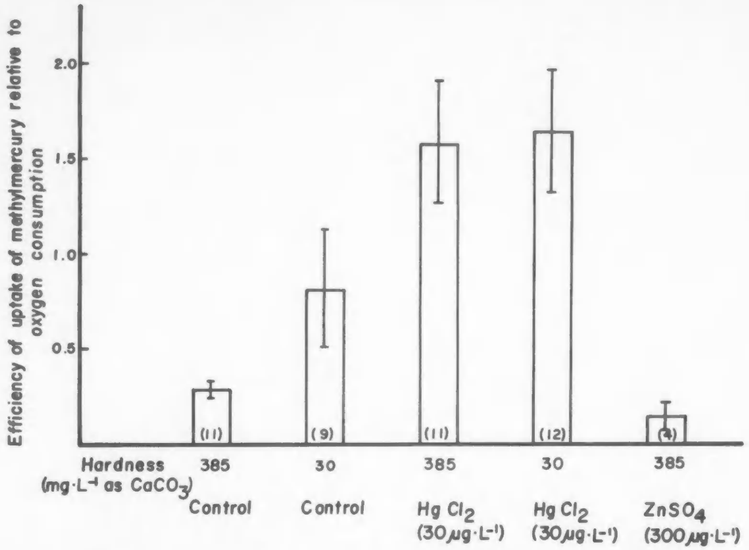

FIG. 1. Efficiency of uptake of methylmercury relative to oxygen of rainbow trout, *Salmo gairdneri*, in relation to water hardness, inorganic mercury, and zinc. Bars indicate mean ± 95% confidence limits; sample sizes are in parentheses.

not required for uptake of methylmercury across nonrespiratory surfaces or depuration of methylmercury before counting. At the concentrations of labeled methylmercury and cold mercuric chloride that we employed, isotopic exchange of labeled mercury is less than 0.5% (Kudo et al. 1977).

The efficiency of methylmercury uptake relative to oxygen, $E(p/o)$, was estimated as

$$E(p/o) = (\Delta P/[\mathrm{P}])/(Q/[\mathrm{O_2}])$$

where ΔP is the rate of methylmercury uptake of the trout (nanograms CH_3Hg per hour), [P] is the average methylmercury concentration (nanograms CH_3Hg per millilitre), Q is the rate of oxygen consumption (milligrams O_2 per hour), and $[O_2]$ is the mean oxygen consumption (milligrams O_2 per millilitre).

Uptake efficiencies were analyzed using the appropriate analysis of variance model. Cell means were compared using the least significant difference (LSD) method if the F ratio was significant ($P < 0.05$). The log transformation was employed, as inspection of residual and normal probability plots indicated that this transformation was required to comply with statistical assumptions of normality and homogeneity of variance.

Results and discussion — Respiration of the trout was not affected by the concentrations and duration of exposure to labeled methylmercury, mercuric chloride, and zinc sulphate employed. Oxygen consumption of the fish for the interval in which labeled methylmercury and metals were added was not significantly different from the preceding interval (paired t-test, $P > 0.05$). The measured concentrations of methylmercury ($<5\ \mu g \cdot L^{-1}$), mercuric chloride ($\sim 30\ \mu g \cdot L^{-1}$) and zinc sulphate ($\sim 300\ \mu g \cdot L^{-1}$) were less than 10% of the 96-h LC50 of these compounds to salmonids (Matida et al. 1971; McKim et al. 1976; Spear 1981). Our observation that oxygen consumption was not significantly influenced by these concentrations of methylmercury, mercuric chloride, and zinc sulphate is consistent with a previous study in which similar concentrations of methylmercury also did not significantly affect oxygen consumption (Rodgers and Beamish 1981).

The efficiency of methylmercury uptake of the trout increased significantly ($P < 0.05$) in soft water or when inorganic mercury was added but decreased with the addition of zinc (Fig. 1). In hard water, without added metals, the $E(p/o)$ of 0.28 ± 0.02 (mean ± SE, $N = 11$) was similar to the value of ~0.25 previously obtained for trout in the same water at 10 and 20°C (Rodgers and Beamish 1981). In soft water without added metals, the $E(p/o)$ increased approximately threefold to 0.82 ± 0.13 ($N = 12$). When mercuric chloride was added with labeled methylmercury, the $E(p/o)$ increased to ~1.6 in both hard and soft waters (1.57 ± 0.15, $N = 11$, and 1.64 ± 0.15, $N = 12$, respectively). In hard water, when zinc sulphate was added with labeled methylmercury, the $E(p/o)$ decreased slightly but significantly to 0.15 ± 0.02 ($N = 4$) relative to values of 0.28 obtained without added zinc.

Changes in the efficiency of methylmercury uptake in hard and soft waters are consistent with the effects of ambient calcium concentration on gill permeability and electric potential. In freshwater teleosts, gill permeability varies inversely with calcium concentration (Ogawa 1974; Isaia and Mansoni 1976; McWilliams and Potts 1978). Although the mechanisms through which calcium affects gill permeability are poorly understood, calcium influences fluxes across the gills of both nonpolar molecules (tritiated water) and ions (Na^+, H^+ and Cl^-), and changes in both active and passive transport processes are implicated (Ogawa 1974; Isaia and Mansoni 1976; McWilliams and Potts 1978). In addition, the electrical charge of the gill becomes more positive at higher calcium concentrations (McWilliams and Potts 1978); consequently, uptake of cations, such as CH_3Hg^+, should decrease in waters of elevated calcium concentration. The observed changes in uptake of methylmercury with water hardness would thus seem to result from Ca^{2+}-induced changes in gill permeability and charge. Although changes in water hardness also involve a slight decrease in pH, at pH greater than 6.0, pH had little effect on gill permeability or charge (McWilliams and Potts 1978). Further research using reconstituted water of defined calcium concentration, alkalinity, and pH could facilitate isolation of the effects of the specific components of water hardness.

The increased efficiency of methylmercury uptake relative to oxygen in soft water is consistent with and may in part explain the common observation of decreased toxicity of metals in hard water (Sprague 1970). Unfortunately, data on the effects of water hardness on toxicity of waterborne methylmercury are not currently available.

The increased efficiency of methylmercury uptake in soft water is also consistent with the observation that fish from lakes of low alkalinity and pH contain higher concentrations of methylmercury than fish from adjacent waters of higher alkalinity and pH (Jernelov et al. 1975; Scheider et al. 1979). Our results would suggest that the higher methylmercury residues in fish from softwater lakes could result from the effects of ambient Ca^{2+} on uptake of waterborne methylmercury. The effects of pH on methylmercury uptake by fish have yet to be defined.

Inorganic mercury is usually present in natural waters in considerable excess of methylmercury (Kudo et al. 1982). Thus, the increased efficiency of methylmercury uptake relative to oxygen in the presence of inorganic mercury may be of considerable environmental significance. The similarity in the efficiencies of methylmercury uptake relative to oxygen in hard and soft waters when mercuric chloride was added may indicate a saturation of uptake. Short-term exposure to concentrations of mercuric chloride as low as 12 $\mu g \cdot L^{-1}$ increased mucus production of rainbow trout (Lock and van Overbeeke 1981). Mercuric chloride has also been reported to change the structural properties and permeability of trout mucus (Varanasi et al. 1975). Changes in the rate of production and structural properties of the mucus covering the gill epithelium may thus account for the increased efficiency of methylmercury uptake with coincident exposure to mercuric chloride. Sublethal concentrations of zinc have also been reported to increase mucus production in trout (Sellers et al. 1975), yet the addition of zinc with labeled methylmercury decreased rather than increased the efficiency of methylmercury uptake relative to oxygen. The mechanisms by which inorganic mercury and zinc affect uptake of waterborne methylmercury thus remain undefined. The reduction in the efficiency of methylmercury uptake with the addition of inorganic zinc may indicate a competition for sites of uptake on the gill. This interpretation is consistent with observations that another divalent metal, copper, was antagonistic to methylmercury in toxicity tests with the blue gourami, *Trichogaster trichopterus* (Roales and Perlmutter 1974).

The correspondence between the increase in the efficiency of methylmercury uptake in soft water and field reports of elevated mercury concentrations in fish from lakes of low alkalinity, hardness, and pH, demonstrates that laboratory experiments can be useful in corroborating and explaining the dynamics of methylmercury in natural environments. Likewise, the conundrum of high mercury concentrations in fish from waters without apparent sources of added mercury may be amenable to a similar approach. A detailed comparison of the chemical composition of these waters could reveal common factors. The effects of these common factors on uptake of waterborne methylmercury could then be examined.

Our experiments have shown that the efficiency of methylmercury uptake relative to oxygen is sensitive to changes in ambient water quality. This ratio should thus prove useful in isolating the effects of specific environmental entities on uptake of waterborne methylmercury. Conversely, comparison of the efficiency of uptake relative to oxygen for different xenobiotics under standard conditions may provide a sensitive standard index of the potential for these compounds to be accumulated from water by fish.

Acknowledgments — We thank E. Thomas for technical assistance and R. W. Bradley for zinc analysis. Research was supported by a strategic grant from the Natural Sciences and Engineering Research Council of Canada.

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BOOK REVIEWS/COMPTES RENDUS

Philopatry, Inbreeding, and the Evolution of Sex. *W. M. Shields. State University of New York Press, Albany, New York 12246, USA. 1982. ix + 245 p. $49.00 cloth, $16.95 paper.*

The high cost of sex has been of concern to theoreticians for some time. Despite their concern and a number of papers and books on the subject, there has not been a completely satisfactory or totally convincing argument to account for the evolution of sex, since there appears to be no obvious compensation for the presumed cost of sex. This book attempts to resolve this dilemma by addressing the three topics of the title, in terms of their adaptive functions.

Basically Shields poses the question, "Why philopatry?," i.e. why do so many animals (and plants), that could apparently do otherwise, return to (or remain in) the area of their birth to breed? This necessarily expands to include the beneficial and deleterious consequences of inbreeding. He concludes by viewing the evolution of sex, in light of his considerations on philopatry and inbreeding. The author himself points out that many of these notions are not new, and in fact, acknowledges his particular dependence on, and close similarity to, earlier arguments in population genetics advanced by Sewall Wright. Shields' contribution is to suggest that, (a) philopatry is probably more common and widespread than expected, (b) inbreeding is not only common, but likely does not have the supposed deleterious effects most have assumed in the past, and (c) sexual reproduction appears to have evolved because the cost of inbreeding is less than the segregational load of outbreeding or the mutational load of asexuality. Sexual reproduction, coupled with inbreeding, is a compromise between the disruptive effects of (sexual) outbreeding and the inflexible, channelized effects of asexuality. As the author states, sex becomes a paradox lost.

This book will certainly be of interest to theoreticians, but even practically oriented biologists should read at least parts of it. Those concerned with stock concepts in fisheries, for example, will no doubt be interested in the statements and conclusions concerning home site tendencies and the extent of inbreeding. Those concerned with problems of biogeography will similarly want to read these parts of the book as well. There have been a number of recent developments, for example hypotheses concerning fish stocks and reproductive strategies in fishes, relevant to the arguments raised in this book but not considered by the author. As is often the case with theoretical considerations, however, sweeping conclusions are based upon rather limited considerations of (often) secondary sources. The prediction of a negative correlation between fecundity and inbreeding is judged to be upheld for fishes, apparently based upon consideration of four families, with Harden Jones' (1968) book as the reference source. Few would dispute the value of Harden Jones' book, but it is not a primary source, and a good many fish have passed under the bridge since 1968. The author perhaps does himself a disservice in such cases by not considering more recent, extensive literature on the topic. He also opens the way for aspiring critics (or supporters) to test his generalizations by a more thorough review of the primary literature. I am particularly concerned about Shields' tendency towards such generalizations in light of his statement (p. 25–26) that ". . . . a single exception cannot overturn an otherwise useful hypothesis . . . " in a discussion of apparently contradictory evidence. But to the author's credit, most of his generalizations and conclusions are stated as hypotheses, with testable predictions.

It will be a pleasant shock to many biologists that by his own admission Shields' treatment of his subject is not at all mathematical. His admission of mathematical inability may well indicate the situation of too many of us, but is refreshing for its candor and for its occurrence in this particular area of theoretical biology. This should not be construed as a rationalization of ineptitude or support for less than rigorous considerations of theoretical arguments. Presumably any serious theoretical argument (even in biology) can be rendered in rigorous mathematical terms. The problem often seems to be that the mathematics proceeds in advance or even in the absence of the biology, and biologists may be too easily led by the pied pipers of numbers. Shields' book will prove to be worthwhile if it provokes both the mathematically inclined and the field biologists to test his predictions.

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(R448)

Fishes: An Introduction to Ichthyology. *Peter B. Moyle and Joseph J. Cech Jr. Prentice-Hall Inc., Englewood Cliffs, NJ. 593 p. $29.95 (U.S.).*

A basic background in fish systematics anatomy and distribution integrated with recent developments in ecology, physiology, behavior, and evolution is the ambitious aim of this 600-page volume. While the overall accomplishment falls well short of what is probably an impossible task anyway, it is nonetheless worthwhile in certain respects. As a summary of recent work it will serve as a timely update in a field with surprisingly few general texts. Because it is designed as a one volume encyclopedia it suffers the inescapable fate of superficiality with too many topics too thinly covered. Even so if one supposes that the section on form and function was deliberately restricted to one third of the text, then it must be regarded as a very competently written precis. After the facts are summarized there really isn't much room for integration, interpretation and speculation and thus of providing "some feeling for the excitement being engendered by recent research."

The 152-page section on fish diversity is an articulate and effectively illustrated factual summary. Unfortunately the distribution of effort is skewed with too little emphasis on fossil forms and non-teleosts and too much (relatively) on teleosts. For example the 15 pages on sharks, skates and chimaeras (including 5 full pages of illustrations) clearly underrates the importance of this group especially when the text is intended for upper-year university students.

A 52-page treatment of zoogeography is likewise a useful factual summary but, given the nature of the subject, remarkably short on interpretation. While the role of continental drift on fish distribution and evolution is considered the impacts of Pleistocene glaciations are virtually ignored. The 150-page section on ecology is chiefly a habitat catalogue preceded by an introduction which considers some general ecological ideas. The importance of recent work is best illustrated here where much of the material covered has been reported during the last 10–15 years.

Measured against similar texts the Moyle and Cech volume is in many ways a more balanced approach than for example Lagler et al. (1962), *Ichthyology*, even though its coverage is similarly encyclopedic. Precisely because it is encyclopedic the new volume lacks the character and interpretative strength of Marshall's (1965) *Life of Fishes*. This comparison parallels historically persistent differences between most American and British vertebrate zoology texts. The latter emphasize a framework of interpretive and integrative ideas within which factual information is explored and evaluated, a decidedly synthetic approach. American texts emphasize broad, factual reporting often with only the most tentative, small scale interpretation, an encyclopedic approach. In upholding this tradition Moyle and Cech have, in my opinion, shortchanged university and other students of fishes. In summary, this new volume is long on facts and short on ideas.

LAGLER, K. F., J. E. BARDACH, AND R. MILLER. 1962. Ichthyology. John Wiley and Sons, New York, NY. 545 p.

MARSHALL, N. D. 1965. Life of fishes. London Weidenfeld and Nicolson. 402 p.

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(R416)

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Synopsis and Classification of Living Organisms. *Editor in chief Sybil P. Parker. McGraw Hill Book Co., New York. 1982. Two Volumes, xviii + 1166 p., 1232 p. $149.50 (U.S.).* This multi-authored two-volume treatise surveys the entire classification of the animal kingdom and will form the basic reference for years to come. [R479]

Canadian Atlantic Offshore Fishery Atlas. *Edited by D. J. Scarratt. Canadian Special Publication of Fisheries and Aquatic Sciences 47 (Revised). Government of Canada, Fisheries and Oceans, Ottawa. 1982. 101 p. $8.00 (Canada); $9.60 (other countries).* Also available translated into French: *Scarratt, D. J. [éd.]. 1982. Atlas des pêches hauturières de l'Atlantique canadien. Publication spéciale canadienne des sciences halieutiques et aquatiques 47F: 101 p.* The Atlas comprises a series of maps showing the distribution of various Canadian Atlantic commercial fishery species and other important marine resources, together with written descriptions of life history and biology appropriate to an understanding of their environmental sensitivity. [R480]

Manuel d'élevage d'invertébrés dulçaquicoles choisis. *Rédaction par S. G. Lawrence. Publication spéciale canadienne des sciences halieutiques et aquatiques 54F. Gouvernement du Canada, Pêches et Océans, Ottawa. 1982. 181 p. Cette publication est distribuée gratuitement par l'Institut des eaux douces, Winnipeg, Manitoba.* Traduction française de la publication spéciale de S. G. Lawrence intitulée "Manual for the Culture of Selected Freshwater Invertebrates" publiée en 1981. [R481]

Multispecies Approaches to Fisheries Management Advice. *Edited by M. C. Mercer. Canadian Special Publication of Fisheries and Aquatic Sciences 59. Government of Canada, Fisheries and Oceans, Ottawa. 1982. 169 p. $14.95 (Canada); $17.95 (other countries).* This comprises the proceedings of an international workshop convened at the Northwest Atlantic Fisheries Centre in St. John's, Newfoundland, November 26–29, 1979. The purpose of the workshop was to discuss the results of multispecies research, its applications to management, and to consider future research directions. [R482]

Age Determination Methods for Fishes Studied by the Groundfish Program at the Pacific Biological Station. *Doris E. Chilton and Richard J. Beamish. Canadian Special Publication of Fisheries and Aquatic Sciences 60. Government of Canada, Fisheries and Oceans, Ottawa. 1982. 102 p. $7.00 (Canada); $8.40 (other countries).* Although the techniques used to estimate the ages of groundfish species are applied to species off Canada's west coast, they should be applicable to other marine and freshwater species from temperate and arctic waters. Some of the described and illustrated procedures, which include routine identification of annuli on scales, otoliths, otolith sections, fin-ray sections, and spines, are experimental and may be later revised. [R483]

British Columbia Pelagic Marine Copepoda: an identification manual and annotated bibliography. *G. A. Gardner and I. Szabo. Canadian Special Publication of Fisheries and Aquatic Sciences 62. Government of Canada, Fisheries and Oceans, Ottawa. 536 p. $17.95 (Canada); $21.55 (other countries).* Lists 121 pelagic marine net zooplankton of British Columbia waters. Species descriptions summarize synonymies, morphology, distribution, life history, and include ecologic information, present taxonomic status, and references. There is an annotated bibliography. [R484]

Proceedings of the Symposium on Recent Benthological Investigations in Texas and Adjacent States. *Edited by Jack R. Davis. Aquatic Sciences Section, Texas Academy of Science, 11204 Brunt Drive, Austin, Texas 78758. 1982. 278 p. $8.50 (U.S.) includes shipping charges to USA and Canada, add $2 for other addresses.* The Symposium was held at the Texas Academy of Science Annual Meeting 4–6 March 1982. Evaluation of the aquatic biota is recognized as a valuable tool in assessing water quality. Characteristics of the benthos reflect environmental conditions at the time of sampling as well as during the recent past. Benthic species occupy virtually all trophic levels in aquatic ecosystems. [R485]

The New Alchemy Back Yard Fish Farm Book: growing fish in floating cages. *William McLarney and Jeffrey Parkin. Brick House Publishing Company, Andover, Massachusetts. 1982. 77 p.* A nontechnical primer on small-scale aquaculture offering information on the cultivation of fish as a low cost, high quality protein food. [R486]

Fisheries Ecology. *Tony Pitcher and Paul J. B. Hart. The Avi Publishing Company, Inc., Westport, Connecticut. 1982. 414 p. $32.50 (U.S.); $36 (other countries in North and South America).* This book is concerned with the ecology of exploited fish populations on a worldwide basis for freshwater and marine fisheries. It is a valuable text for those involved in management and research. The authors describe fish as components of delicately balanced ecosystems in which ecology is directly connected with the daily activities of man. Discussions include adaptive physiology and behavior of fish, and production and economics of fisheries. [R487]

Acid Rain/Fisheries: proceedings of an international symposium on acidic rain and fishery impacts on northeastern North America. *Edited by Raymond E. Johnson; compiled under the direction of Terry A. Haines, Chairman. Northeastern Division, American Fisheries Society, Bethesda, Maryland. 1982. 357 p. $25.00 (U.S.).* At the symposium held at Cornell University, Ithaca, New York, August 2–5, 1981, speakers summarized the current understanding of the formation of acid precipitation, the effects of acid deposition on surface water quality, and the cause–effect relations of acidification on aquatic organisms in general. This proceedings volume identifies future research needs and contributes to the debate regarding actions needed to deal with this international problem. [R488]

Climate and Fisheries. *D. H. Cushing. Academic Press Inc. (London) Ltd., London. 1983. United States edition published by Academic Press Inc., New York in 1982. 373 p. $49.50 (U.S.); £28 (U.K. only).* Fisheries biologists are interested in variation of stock size with time and have made a connection with climatic change. Wind strength and direction, heating, and light penetration vary with time and these influence fish production, partly because they determine the quantity of food in the plankton. The author describes the long-term changes in fisheries together with the events that lead fishermen to expect dramatic rises and falls. Particular ecosystems are covered including in the North Sea, the Baltic, and off Peru. This book will be of interest to fisheries biologists, climatologists, and ecologists. [R489]

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Canadian
Journal
of Fisheries
and Aquatic
Sciences

Journal
canadien
des sciences
halieutiques
et aquatiques

CJFSDX40(6) 671–834 (1983)
ISSN 0706-652X

Volume 40, No. 6, June 1983

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